Environmental Factors Affecting Pre-Maturity *Alpha*-Amylase Activity in Winter Wheat (*Triticum aestivum*)

Thesis

Environmental Factors Affecting Pre-Maturity *Alpha*-Amylase Activity in Winter Wheat (*Triticum aestivum*)

By

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A thesis submitted to the Open University for the degree of Doctor of Philosophy

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1999

DECLARATION OF AUTHORSHIP

This thesis has been written by myself and describes my own studies, except were due reference is made to the contribution of others.The text of this thesis contains no material which has been submitted for a degree at any other educational establishment.

Bernard J. Major

The search for truth is one way hard and another easy. For it is evident that no one can can master it fully or miss it fully. But each adds a little to our knowledge of nature, and, from all the facts assembled there arises a certain new grandeur.

Aristotle(384-322 B.C.)

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Abbreviations

AB	ADAS Bridgets
ABA	Abscisic acid
ADAS	Agricultural Development and Advisory Service
a.i.	Active ingredient
ANOVA	Analysis of variance
°C-days	Degree Celsius-days
CAP	Common Agricultural Policy
CCFRA	Campden and Chorleywood Food Research Association
CV	Coefficient of variation
DAA	Days after anthesis
df	Degrees of freedom
cDNA	Complementary deoxyribonucleic acid
EU	European Union
FDB	Fluorescein dibutyrate
GA	Gibberellic acid
GATT	General Agreement on Tariffs and Trade
HA	Harper Adams University College
HFN	Hagberg falling number
H-GCA	Home-Grown Cereals Authority
IEF	Isoelectric focusing
ITCF	Institute Techniques des Céréales et des Fourrages
LN	Liquefaction number
mEU g^{-1} dw	Milli enzyme units activity per gram dry weight
mRNA	Messenger ribonucleic acid
Max	Maximum
Min	Minimum
NAO	North Atlantic Oscillation
NIAB	National Institute of Agricultural Botany
P	Probability
PEG	Polyethylene glycol
PHS	Pre-harvest sprouting
pI	Isoelectric point
PMAA	Pre-maturity *alpha*-amylase activity
PoMS	Post-maturity sprouting
PrMS	Pre-maturity sprouting
r.p.m	Revolutions per minute
RPAA	Retained pericarp *alpha*-amylase activity
SB	Sutton Bonington (University of Nottingham)
SED	Standard error of the difference of means
UA	University of Aberdeen
UK	United Kingdom
WOBM	Wheat orange blossom midge
ZGS	Zadoks' growth stage

Acknowledgements

I would like to thank the following for their help during the course of this project, without whom it would not have been possible :-

The Home-Grown Cereals Authority (UK) and the Institut Technique des Céréales et des Fourrages (France) for providing the funding for this project.

Studley College Trust (UK) for awarding me funding to enable my attendance at the 8 th International Symposium on Pre-harvest Sprouting in Cereals held in Detmold, Germany in 1998.

Mr Peter Kettlewell (Harper Adams University College) for his invaluable advice, encouragement and supervision throughout the course of this project.

Dr Gavin Lunn (University of Nottingham) for his supervison, insight and understanding into the practicalities of the project and general affability.

Andy Joynt, Mark Singleton and Mike Warden, for the routine agricultural management of the field experiment site at Harper Adams between 1993 and 1996.

Dave Farmer for his care and maintenance of the plants in the glasshouse before their use in the controlled-environment cabinet experiments.

Adrian Marsh (ADAS Bridgets) for providing field experimental data from ADAS in 1995 and Jon Doney (ADAS Bridgets) for providing data in 1996. Jo Learmonth (University of Aberdeen) for providing field experimental data from Aberdeen in 1995 and 1996.

Chris Wade for help with sampling the field experiment at Harper Adams in the summers of 1994 and 1995 and Jon Chadwick for help with sampling the field experiment in the summer of 1996.

Sam Hawkey for instruction on the use of the Skalar *alpha*-amylase autoanalyser. Gavin Boulby for instruction on use of the HFN machine and the other standard grain quality measurements. John Head, Alison Pearce, Suzanna Lovell and Steven Ravenscroft for performing several of the field experiment *alpha*-amylase, HFN and FDB assays.

All other library, computer and technical staff at Harper Adams who have contributed, and helped make this thesis possible.

Kalpna Kotecha (Campden and Chorleywood Food Research Association) for carrying out some initial *beta*-limit dextrin gel assays, and Nick Saunders and Rachel Seamer for demonstrating the Phadebas gel assay technique.

Savitri Abeyasekera (University of Reading) for statistical advice on data analysis.

Dr Alex Hilton and Dr Ivan Grove for putting up with me in PP3 and with whom it was a great privilege to share an office with. Similarly all former residents at No 17.

Finally, all members of Harper Adams Staff 5-a-Side Football Team and Market Drayton Hockey Club who provided alternative distractions to research.

‘ they have sold’em growed wheat, which they didn’t know was growed, so they say, till the dough ran all over the ovens like quicksilver; so that the loaves be as flat as toads, and like suet pudden inside ’

In ‘ The Mayor of Casterbridge’ by Thomas Hardy 1886.

Abstract

Pre-maturity *alpha*-amylase activity (PMAA) in the absence of sprouting is one of four causes of low Hagberg falling number (HFN) in UK winter wheat (*Triticum aestivum*), reducing the quality and value of milled flour. Other causes include the retention of pericarp *alpha*-amylase activity (RPAA), pre-maturity sprouting (PrMS) and post-maturity sprouting (PoMS).This thesis investigated the effects of environmental factors on PMAA which currently occurs in a variable and unpredictable fashion under UK weather conditions.

A multi-site field experiment on four cultivars (Haven, Hornet, Pastiche and Riband), at four sites (Harper Adams University College, University of Nottingham, University of Aberdeen and ADAS-Bridgets) between 1994-1996 was undertaken to:- establish the frequency of the causes of low HFN; examine the relationship between grain drying-rate and PMAA; determine if it was possible to predict combine harvest HFN.

A range of techniques including a visual sprouting assessment, fluorescein dibutyrate staining, iso-electric focusing and a *beta*-limit dextrin gel and iodine staining test were used to allow the cause of low HFN to be established. Of the forty crops analysed, 22 cases (45%) had detectable amounts of *alpha*-amylase activity. PMAA was identified solely in 2 cases (5%), in combination with PoMS in 8 cases (20%), in combination with RPAA in one case (2%), with PoMS occurring solely in 11 cases (28%). The HFN fell below the breadmaking standard of 250 s in 18 of the 36 site x year x cultivar combinations analysed.This was attributed solely to PMAA in two cases (11%), a combination of PMAA and PoMS in a further eight cases (44%) and solely to PoMS in eight cases (44%). There were no cases where PrMS or RPAA reduced the HFN to below 250 s.

The hypothesis that PMAA is related to the grain drying-rate between 40-20 % moisture content was tested. Grain drying-rate was determined by linear regression analysis using moisture content measurements made at regular intervals during grain development. In site x year x cultivar combinations where PMAA was detected the grain drying-rate was significantly ($P = 0.047$) lower (mean = 1.90 *cf.* 2.30% moisture loss day^{-1}), suggesting a slow grain drying-rate enhances PMAA. However, the low frequency of occurrence of PMAA in isolation prevented quantification of this relationship.

Initiation of PMAA in the grain, was shown to occur from a grain moisture content of 47.8%. A pre-harvest sample taken by hand at 850 °C-days (35 % moisture, Zadoks growth stage 85-87) was shown to enable a prediction of combine harvest HFN to be made in the absence of subsequent rainfall and PoMS. The 95 % confidence limits associated with this HFN prediction were however wide.

The hypothesis that transient changes in temperature early in grain development may affect PMAA, before the onset of any grain drying-rate effects, was tested in five controlled-environment cabinet experiments. Of 36 cultivar x time of transfer combinations undertaken from a 16 / 10 °C to a 26 / 20 °C temperature regime, six led to a significant increase ($P < 0.05$) and one led to a significant decrease in PMAA. Of the 18 cultivar x time of transfer combinations undertaken from a 25 / 20 °C to a 16 / 10 °C temperature regime, one led to a significant increase and one led to a significant decrease in PMAA. A comparison between the field and controlled-environment experiment results highlighted that after conditions putatively stimulating PMAA had been encountered, subsequent environmental factors, such as mean temperature and relative humidity may also affect PMAA.

It was concluded that PMAA can be enhanced by transient increases in temperature before the grain reaches 40% moisture content and by a slow grain drying-rate between 40-20% moisture content. The variability in the results, however, also suggested other environmental factors were influencing PMAA.

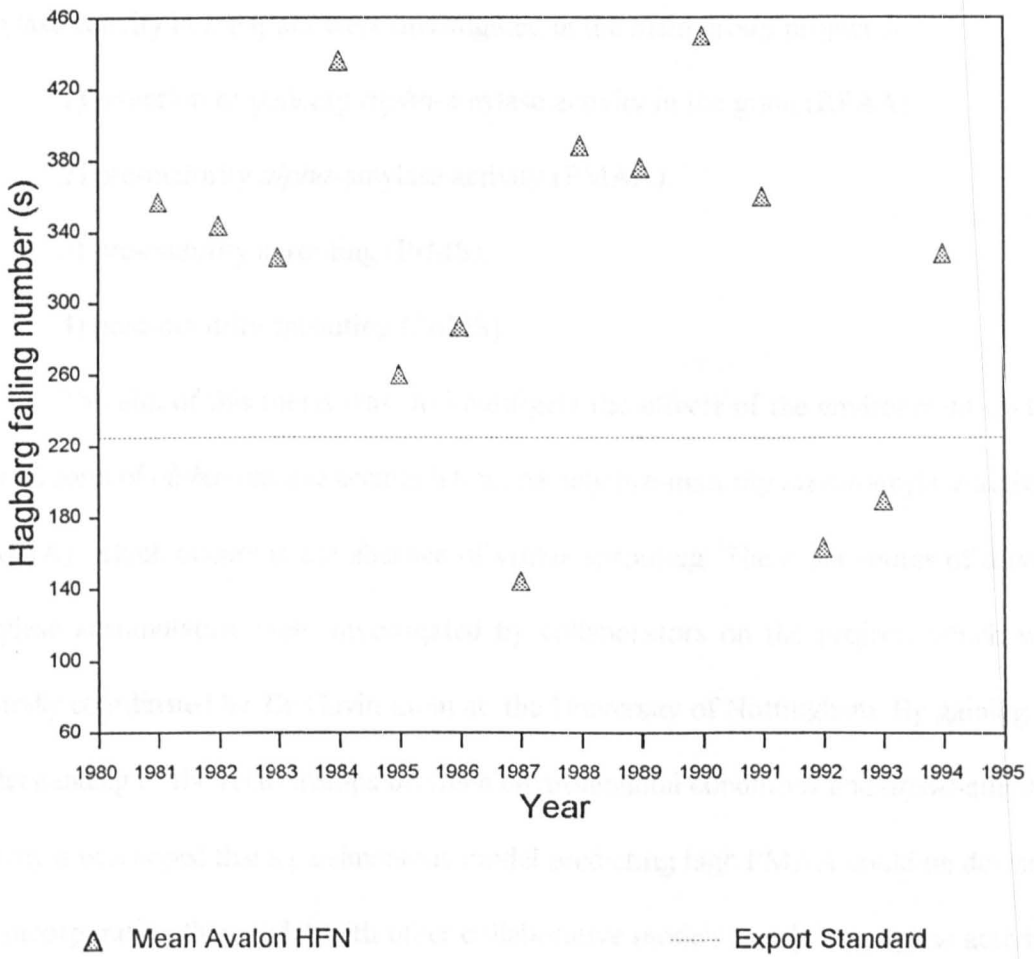
1. Background

The work in this thesis formed part of a larger four-year group project funded by the Home-Grown Cereals Authority (H-GCA, UK) and the Institut Technique des Céréales et des Fourrages (ITCF, France) entitled: 'Physiological Control of Hagberg Falling Number (HFN) and Sprouting in Winter Wheat and the Development of a Prediction Scheme'. This project involved collaboration between the following UK institutions; Harper Adams University College (Newport, Shropshire); University of Nottingham (Sutton Bonington, Leicestershire); University of Aberdeen (Aberdeen, Scotland); Agricultural Development and Advisory Service (ADAS-Bridgets, Winchester, Hampshire) and the Institut Technique des Céréales et du Fourrages (Paris, France).

HFN is currently the major quality restraint restricting the consistent and increased use of home-grown grain. A typical minimum HFN specification of 220-250 s is required for grain destined for export and breadmaking. UK cultivars grown for these markets typically fail to reach these specifications about one year in four (See Figure 1.1). This intermittent problem has serious effects on grain quality and major financial repercussions for growers, the milling and baking industry, the animal feed sector and exporters.

The overall aim of the group project was to develop a scheme enabling the reliable forecasting of the risk of pre-harvest sprouting and *alpha*-amylase accumulation and the associated decline in HFN of wheat in the UK and France. Reliable prediction of the HFN of grain would benefit growers in several ways. It would allow the allocation of combining priorities to be assigned to grain during poor harvesting conditions, thus ensuring the quality of the grain harvested was economically optimised. Additionally, storage requirements could be more prudently planned, thereby avoiding the risk of downgrading grain by mixing grain lots of vastly differing HFN. A prediction scheme may also help the

Figure 1.1 : Variations in the Hagberg falling number (HFN) of the winter wheat cultivar Avalon at Harper Adams University College from 1981-1994.



milling and baking industry, by increasing the uniformity of the harvested crop and also stabilising the supply of high quality HFN home-grown grain from year to year. It would also enable exporters to forecast more accurately the quantities of home-grown wheat likely to meet export specifications, thus allowing more flexible export trading. The scheme would also be of use to the animal feed sector by allowing them to predict more accurately the amount and cost of wheat grain available for incorporation into animal feed.

To produce a prediction scheme requires a precise understanding of the effects of the environment on physiological mechanisms during grain development which can lead

to the generation of unacceptably high *alpha*-amylase activity and low HFN in combine harvested grain. Four possible mechanisms which affect the generation of high *alpha*-amylase activity in the grain were investigated in the main group project :-

1) retention of pericarp *alpha*-amylase activity in the grain (RPAA).

2) pre-maturity *alpha*-amylase activity (PMAA).

3) pre-maturity sprouting (PrMS).

4) post-maturity sprouting (PoMS).

The aim of this thesis was to investigate the effects of the environment on the second form of *alpha*-amylase accumulation, namely pre-maturity *alpha*-amylase activity (PMAA), which occurs in the absence of visible sprouting. The other routes of *alpha*-amylase accumulation were investigated by collaborators on the project, which was centrally coordinated by Dr Gavin Lunn at the University of Nottingham. By gaining an understanding of the relationships between environmental conditions and *alpha*-amylase activity it was hoped that a parsimonious model predicting high PMAA could be devised. By incorporating this model with other collaborative models on *alpha*-amylase activity, it was hoped that an overall scheme could be devised allowing accurate pre-harvest prediction of *alpha*-amylase activity and HFN in wheat. This has proved to be an over ambitious target due to the complexity of the environmental interactions with grain physiology identified in the course of the studies. Nonetheless an operable HFN prediction scheme based on the results obtained from the main project and this thesis has been devised (Lunn *et al.*, 1998).

2. Review of Literature

2.1 Introduction

2.1.1 The importance of wheat quality

The most agronomically important plant group utilised by man is the *Gramineae* family. This simple group of grasses has been cultivated by man over the centuries, principally for its seeds. This has led to the development of today's high yielding cereal cultivars, which form the staple diet of the majority of the world's population (Percival, 1974). Of these cereal cultivars, wheat is the most important as it occupies the largest area of production, producing the highest quantity of harvested grain. The agronomic adaptability, ease of storage, nutritional quality and the ability of its flour to be processed into a variety of products have all led to wheat becoming an important constituent of many peoples diets. Wheat is also an important economic commodity, reflected by the fact that world trade in wheat exceeds the trade in all other cereal grains combined (Hanson *et al.*, 1982). Bread wheat (*Triticum aestivum*) is an important agricultural crop in the UK (Table 2.1) . Of this total production in 1991/92, 29% was used in flour milling, 30 % in animal feed, 33% was exported, 2% was used in seed production, 4% was used in other human and industrial processes and 2 % found other uses (Anon., 1993a).

Table 2.1 : Wheat Statistics in the UK 1991/1992 (Anon., 1992).

Wheat in the UK	Tonnes (1000)	Value (£ million)
Total Production	14363	1689.2
Total Marketed	12970	1527.9
Total Remaining on the Farm	1400	161.3
Wheat Exported	4647	590.2
Wheat Imported	863	168.1

Any factor which affects the yield or quality of the wheat crop will be of economic importance to growers. The significance of these effects are determined by the final market price growers are likely to receive for their crops and the costs involved in trying to reduce the effects of any adverse factor. Traditionally, yield has been perceived by growers as the most important factor determining the profitability of a crop. The quality of the harvested crop is, however, becoming of increasing importance to profitability as the effects of surpluses, the reformed Common Agricultural Policy (CAP) and the General Agreement on Tariffs and Trade (GATT), take effect (DeMaria, 1994).

In some seasons, wheat cultivars grown in the UK are prone to the formation of high *alpha*-amylase activity in the grain before harvesting can commence (Gale and Lenton, 1987). This effect is attributable to the differing environmental conditions experienced between seasons. Pre-harvest deterioration in the quality of the wheat crop can have severe effects on its market value, due to the deleterious effects of high *alpha*-amylase activity on end product quality. This is currently the major factor restricting the increased use of UK grain by millers (Anon., 1993b).

2.1.2 The effect of high *alpha*-amylase activity on the end use of wheat grain

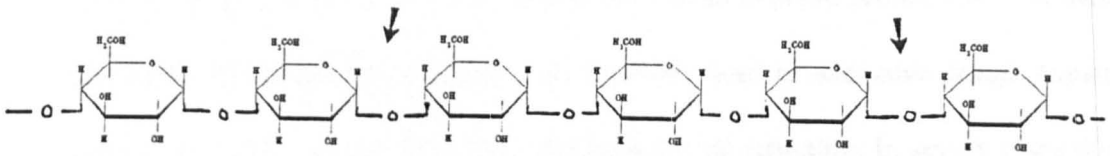
Leavened bread is the most significant wheat flour product which is adversely affected by the presence of high *alpha*-amylase activity in the flour. During the breadmaking process, the addition of water to flour, and the subsequent heating of the dough mixture causes gelatinisation of starch granules. Gelatinised starch is rapidly broken down by *alpha*-amylase which hydrolyses the α-(1,4)-glycosidic bonds in amylose and amylopectin, the high molecular weight glucan polymers making up starch (Hill and MacGregor, 1988). The breakdown products formed are dextrins and maltose, which are further broken down by *beta*-amylase and maltase respectively to form glucose.

Figure 2.1 : Enzymic action of *alpha*-amylase on starch.

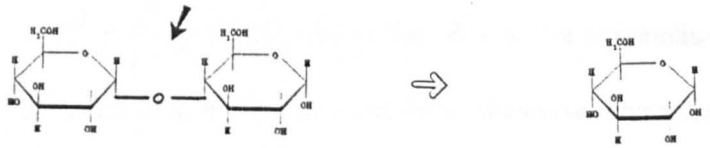
Starch = Amylose + Amylopectin

a) Amylose : α-(1-4) glycosidic bonds

Alpha-amylase acts as an *endo*-acting hydrolase on α-(1-4) glycosidic bonds



α-glucosidase

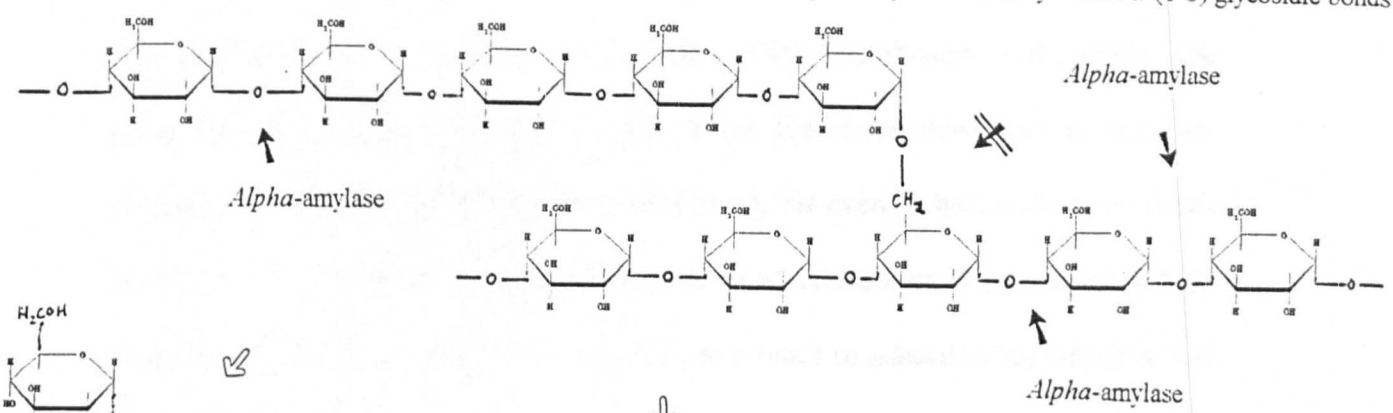


Maltose

Glucose

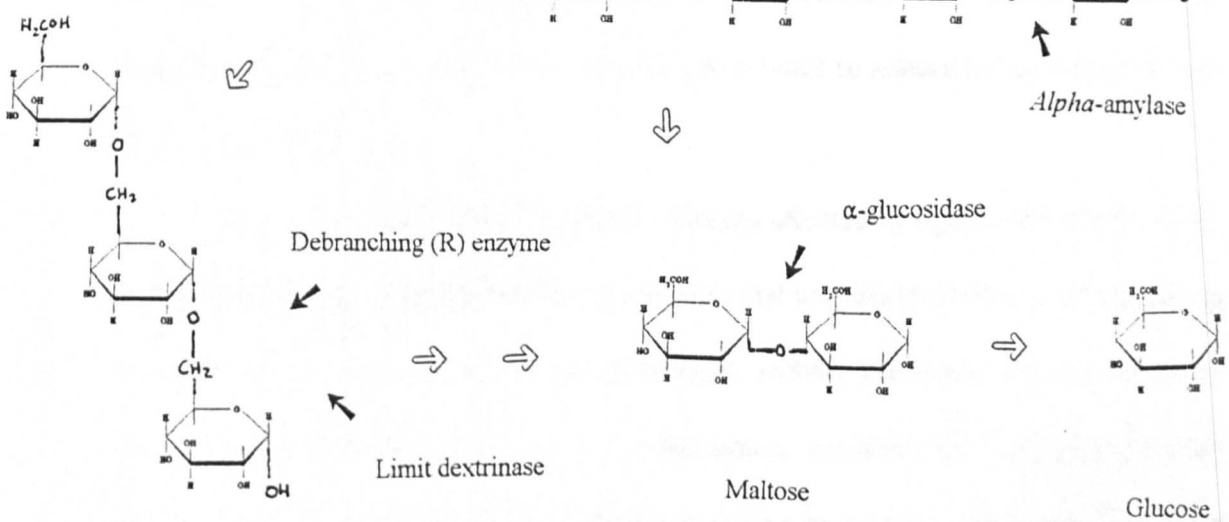
b) Amylopectin : α-(1-4) glycosidic bonds + α-(1-6) glycosidic bonds

Alpha-amylase cannot hydrolase α-(1-6) glycosidic bonds



α-glucosidase

Debranching (R) enzyme



Limit dextrinase

Maltose

Glucose

Limit-dextrins

(Figure 2.1). Glucose is normally fermented by Baker's yeast (*Saccharomyces cerevisiae*), with the consequent generation of carbon dioxide, which causes loaves to rise when the gas is trapped by the visco-elastic gluten protein matrix. Consequently a basal level of *alpha*-amylase activity is required for common bread production. The presence of excess *alpha*-amylase activity can, however, lead to excessive dough expansion, resulting in large volume loaves with a fragile crumb structure. In severe cases this can result in the loaves collapsing. Additionally, the baked loaves tend to be of poor colour and possess an intense crust colouration, due to the caramelisation of reducing sugars, making them unattractive to the consumer. However, the principal problem caused by excess *alpha*-amylase is the generation of excessive amounts of dextrins and sugars which cause a sticky crumb structure to form. Problems are generated on the production line at the high speed slicing blades, as crumb deposits adhere and accumulate on the blades. This leads to a deterioration in the slicing quality of the loaves, with the blades tearing rather than slicing the bread (Buchanan and Nicholas, 1980; Chamberlain *et al.*, 1981). The accumulation of sticky deposits eventually causes the slicing machinery to seize up. Considerable inconvenience is caused to the bakery by this event, which is also very costly in terms of lost production time and marketable product. Poor quality bread made from high amylase flour is noticed by the consumer as it tends to adhere to the teeth and roof of the mouth when eaten.

Other wheat flour products are also adversely affected by high levels of amylolytic activity. Chapattis and flat breads tend to be pale and unattractive when produced using flour with a high *alpha*-amylase content (Derera, 1989a). Cantonese noodles are more severely affected, having a dark unattractive appearance, coupled with a soft eating quality (Orth and Moss, 1987). In starch and gluten extraction high *alpha*-amylase activity leads

to a reduction in starch quality and an increase in unusable soluble carbohydrate compounds (Anon., 1994a).

To summarise, products made from wheat flour containing high *alpha*-amylase activity are generally of an inferior quality, when compared to products made from flour with low *alpha*-amylase activity. The determination of *alpha*-amylase activity in a grain sample is thus of considerable importance in determining the potential end use and market value of a grain consignment. There are several methods for determining *alpha*-amylase activity in flour and its potential effects on bread-making properties. The most widely used method in commercial trading at present is the Hagberg falling number test.

2.1.3 The Hagberg falling number (HFN) test

The HFN test was developed to provide a reasonably rapid and cheap indication of the amount of *alpha*-amylase activity present in the grain (Hagberg, 1960; Perten, 1964). This grain quality measurement is widely employed by grain merchants and millers in the UK. The Hagberg testing equipment is a simple viscometer which measures the time (in seconds) taken for a plunger to fall a set distance through a heated slurry of ground wholemeal grain and water (Anon., 1982). Samples with higher *alpha*-amylase activity will have lower HFN, due to the increased hydrolysis of gelatinised starch in the slurry into sugars and dextrin. This reduces the viscosity of the slurry allowing the plunger to fall more rapidly.

2.1.4 Hagberg falling number (HFN) specifications and their importance

A minimum HFN of 220 s is generally specified for grain destined for breadmaking purposes (Anon., 1993b). Many UK millers specify higher HFN requirements, with values around 250 s not uncommon ; some prefer samples with values >300 s, >350 s where amylolytic activity is supplemented with fungal amylase (Anon., 1993b). With 26.5% of

home-grown wheat used in milling and baking these HFN specifications are very important market criteria (Table 2.2). Unfavourable weather conditions, leading to poor quality UK harvests, often lead millers into lowering these higher HFN specifications. This is for economic reasons, as it allows them to avoid importing larger quantities of higher quality foreign bread-making wheat, which are at present less cost effective to use. In biscuit-making higher *alpha*-amylase activity is tolerable in the production process, thus grain with a minimum HFN of 140 s is often acceptable (Anon., 1993b).

A common basic requirement from many overseas markets is wheat with a minimum HFN of 225 s and a specific weight of 76 kg hl^{-1} (Toft, 1994). The importance of this market is demonstrated by the fact that between 25-33% of the UK wheat crop is exported. Growing cultivars which can satisfy the minimum HFN specifications thus opens up another potential market for the grain. Although there is no generally set HFN specification for export feed wheat, some animal feed buyers do specify an HFN of 200 s, although this is relatively rare (Anon., 1993b). In the European Union (EU) a price support mechanism known as intervention operates as a last resort for the disposal of grain. The EU sets intervention standards for common wheat, with a current minimum HFN specification of 220 s (Anon., 1994b).

Grain not meeting defined HFN specifications and other quality requirements of the above markets generally ends up in the feed wheat market where it faces severe price competition from other grains such as maize. The large price differentials in terms of £ per tonne between bread-making grade, export milling grade and feed grade wheat can have serious economic consequences for a grower whose wheat does not reach the quality specifications of his target market (McMaster, 1987; Wahl and O'Rourke, 1993). With buyers increasingly focusing on wheat quality, specifications for particular markets are

Table 2.2 : Hagberg falling number (HFN) values and specifications.

Hagberg falling number (s)	Specification	[1]NIAB ratings of UK cultivars	Examples of UK cultivars
62	Lowest possible value		
100			
>140	Biscuit-making requirement		
160			
190		Low	Hornet Haven
219			
>220	Intervention Standard	Medium	Riband Brigadier
>225	General Export Standard		
249			
>250	Bread-making Standard	High	Avalon
279			
280		Very High	Soissons Hereward Pastiche
309			
310		Extremely High	Spring Wheats
340			
460-	Top end of range for UK cultivars		

[1]NIAB HFN Ratings of UK cultivars recommended for Growers Use (Anon., 1991).

likely to become more rigorously defined. Achieving these quality standards will become of increasing economic importance to growers.

2.1.5 The relationship between HFN and *alpha*-amylase activity

A relatively good relationship exists between HFN and *alpha*-amylase activity, compared to the relationship between percentage sprouting and HFN or *alpha*-amylase activity . A curvilinear relationship between HFN and *alpha*-amylase activity has been

identified, which can be linearised by expression as a liquefaction number (Perten, 1964):

$$\text{Liquefaction Number (LN)} = \frac{6000}{\text{HFN} - 50}$$

This correlation is less well defined when *alpha*-amylase activity is very low. This is due to the effects of other genetically controlled factors (Finney, 1985) and the starch properties of the grain which are predominant at high HFN (Olered, 1976; Ringlund, 1983). Differences in HFN values for samples with similar *alpha*-amylase activities, have been shown to be related to starch susceptibility to enzymic attack, caused by differences in initial starch gelatinisation temperatures. The rapid rise in temperature in the HFN test means hydrolysis time is a limiting factor. Any small differences in the early gelatinisation behaviour of the starch are thus magnified in the final HFN result leading to the observed differences between samples of similar *alpha*-amylase activity (Corr and Spillane, 1969). Nonetheless the liquefaction number is commonly used by UK millers to produce blends of flour of specific *alpha*-amylase activity from grain lots differing in HFN.

The relative simplicity of the HFN test and its current widespread application, mean that it is likely to remain in use in commercial trading for the foreseeable future as a measure of *alpha*-amylase activity. This is despite concerns over the precision and reliability of results obtained expressed by several authors (Finney, 1985; Vaidyanathan, 1987; Moot and Every, 1990). Thus research focusing on the quality of commercial wheat must consider both HFN and *alpha*-amylase activity, particularly as Mares (1989) states that "while *alpha*-amylase activity is a good measure of weather damage, it cannot be used with confidence to predict falling number accurately".

2.2 Structure and development of the wheat caryopsis/grain

2.2.1 Structure

In order to comprehend the complexity of the processes involved in the production of *alpha*-amylase in the wheat grain it is necessary to understand its morphology and ontogeny. The correct botanical term used to describe the harvested product of the wheat plant, is an indehiscent fruit or caryopsis, although the common term "grain" will be used here. The wheat grain has also been described as a "seed" or "kernel" by other authors. Figure 2.2. illustrates the morphology of the principal tissues in a mature wheat grain.

2.2.2 Grain development

2.2.2.1 Fertilisation

The development of the grain begins when a pollen grain from an anther lands on the stigma of an ovary. A pollen tube then develops, penetrating the stigma and progressing to the embryo sac.

The mature pollen grain possesses two nuclei, one moves down the pollen tube, and divides by meiosis into two male gametes. One gamete then fuses with the egg cell nucleus of the ovary to form the zygote, which will develop into the embryo. At the same time the other male gamete fuses with two polar nuclei in the ovary, to form a triploid primary endosperm nucleus. This later develops into the starchy endosperm and aleurone layer in the mature grain. The seed coat and pericarp develop from the original maternal tissue and therefore have the same chromosomal complement as the maternal plant. Table 2.3 illustrates the chromosomal complement of the various tissues in the mature grain.

Figure 2.2 : Structure of a mature wheat grain bisected longitudinally through the crease (adapted from MacMasters *et al.*, 1971).

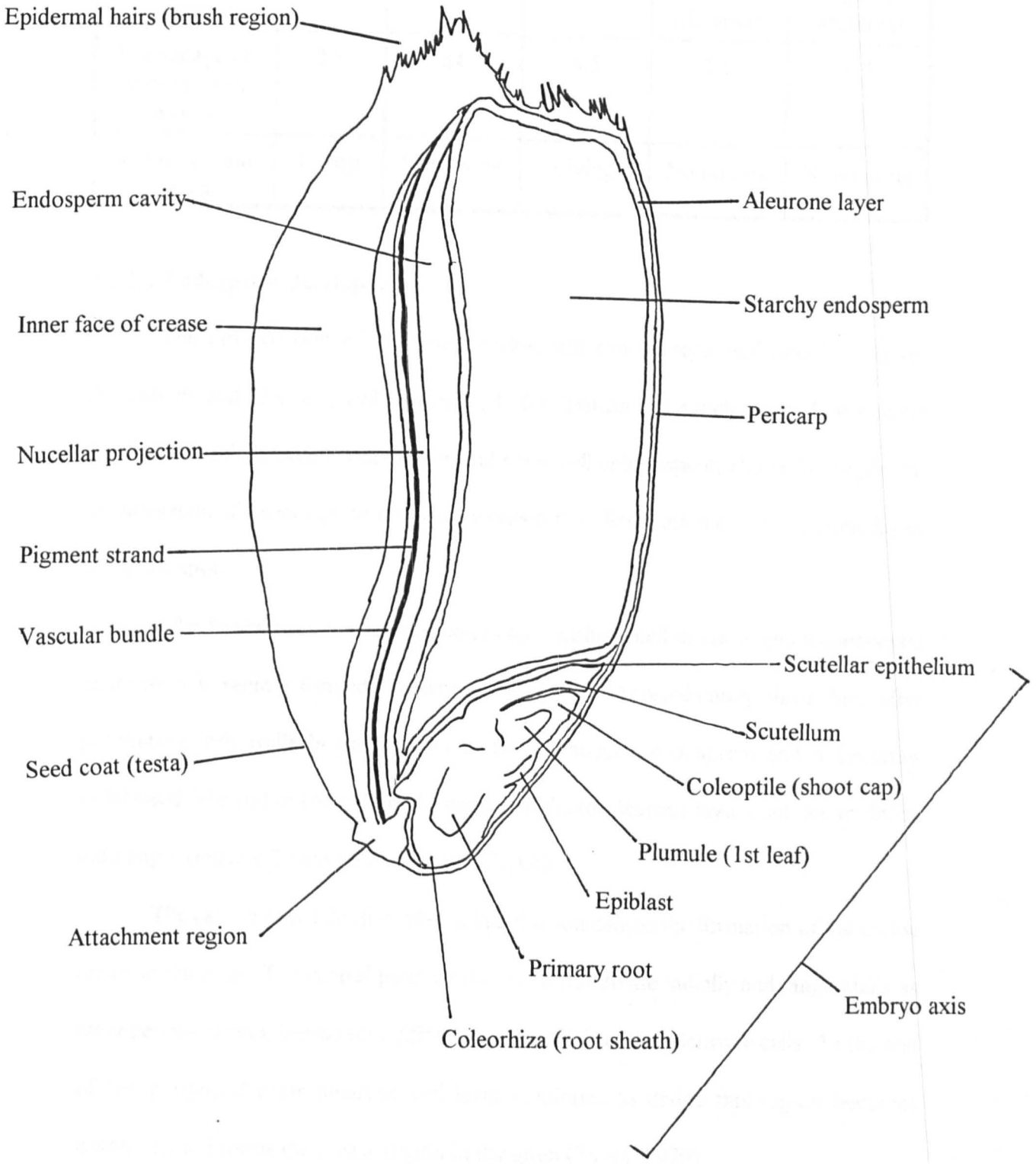


Table 2.3 : Chromosome complement of constituent tissues in wheat grain.

	Embryo	Starchy endosperm	Aleurone	Seed coat	Pericarp
Chromosome complement	Diploid	Triploid	Triploid	Diploid Maternal	Diploid Maternal
Percentage of mature grain weight	2.5	84	6.5	2.5	4.5
Status at grain maturity	Living	Non-living	Living	Non-living	Non-Living

2.2.2.2 Endosperm development

The development of the wheat endosperm can be separated into five phases (Simmonds and O'Brien, 1981) namely; I) fertilisation; II) synchronous free nuclear division ; III) cellularisation, cell division and some cell enlargement; IV) cell enlargement ,accumulation of the storage reserves in the cells and differentiation of the aleurone layer; V) maturation.

After fertilisation, nuclear division occurs without cell division and a coenocytic endosperm is rapidly formed (Briarty *et al.*, 1979). Approximately three days after pollination, cell walls begin to form in the coenocytic endosperm and it becomes cellularised. The endosperm cells in the peripheral proto-aleurone layer continue to divide until approximately 20 days after anthesis (DAA).

The pattern of cell division after cellularisation causes the formation of the crease region in the grain. The ventral peripheral cells do not divide radially and tangentially as other peripheral cells, instead they differentiate into thick walled aleurone cells. As the rest of the peripheral proto-aleurone cell layer continues to divide this region becomes enveloped and forms the crease region in the grain (Evers, 1970).

Cell division then halts, with subsequent increases in grain size due solely to the enlargement of existing cells due to vacuolation (water uptake).The cells also begin to accumulate the storage reserves of the grain namely:- starch, protein and lipids (Jenner *et al.*, 1991). During this stage the proto-aleurone cells in the endosperm develop as a separate, clearly identifiable aleurone layer surrounding the starchy endosperm (Morrison *et al.*, 1978) . Aleurone cells are fully developed by approximately 35 DAA and are characterised by thickened cell wall surrounding a cytoplasm rich in protein (aleurone) granules and lipid droplets, but containing no starch granules.

After approximately 35 DAA, and following the attainment of maximum dry weight, the endosperm begins its maturation phase, which is associated with the drying and loss of water from the grain (Meredith and Jenkins, 1975). This process is completed by 55-60 DAA. At maturity the cells in the starchy endosperm are non-living and filled with starch and protein (Campbell *et al.*, 1981), whereas the surrounding aleurone cells contain no starch but are very much alive and awaiting the "switch" to the germinative mode (Jones and Jacobsen, 1991).

2.2.2.3 Embryo development

The embryo develops within the embryo sac, forming a proembryo. This rapidly differentiates into the scutellum, the coleoptile, enclosing the primary shoot (plumule) and the coleorhiza enclosing the primary root or radicle (Figure 2.3). During development the embryo derives nutrients by hydrolysing adjacent aleurone cells, nucellar parenchyma and endosperm cells which eventually form a 'crushed' cell layer next to the scutellum (Smart and O'Brien, 1983). The scutellum is also believed to regulate endosperm mobilisation during germination by secreting a hormonal message to the aleurone layer (Negbi, 1984).

Figure 2.3 : Structure of a mature wheat embryo bisected longitudinally (adapted from Fahn, 1982).

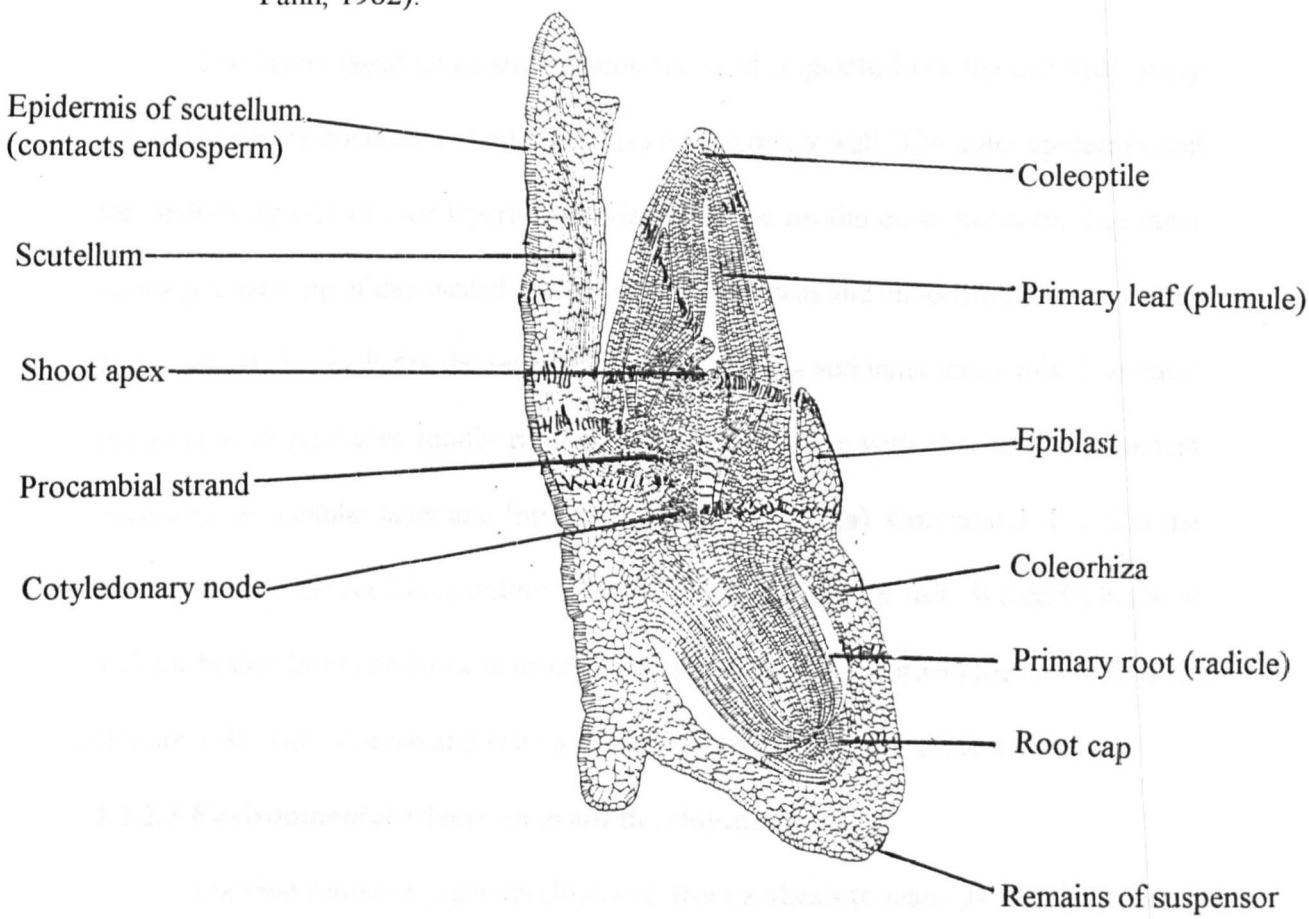
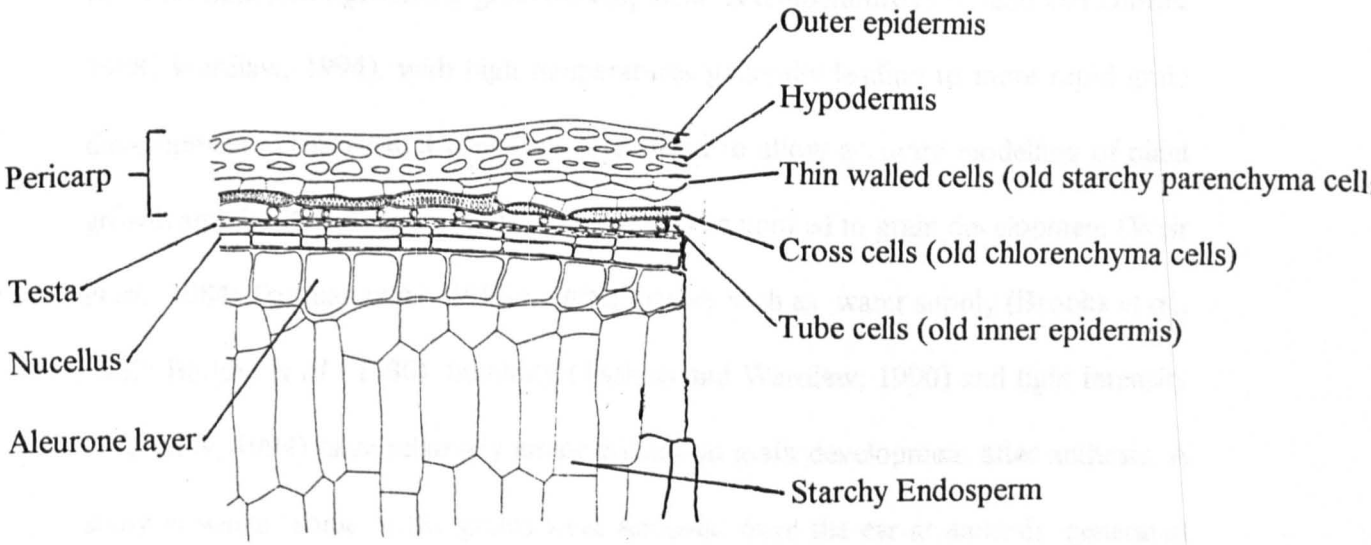


Figure 2.4 : Structure of the mature pericarp, cross sectioned (adapted from Fahn, 1982).



2.2.2.4 The pericarp and seed coat

The layers (seed coat) surrounding the seed originate from the maternal ovary tissue namely the nucellus and integuments and the ovary wall. The outer epidermis and the underlying one or two layered hypodermis make up the outer pericarp. The inner pericarp is made up of thin walled crushed parenchyma cells and underlying cross and tube cell layers. It also includes the remains of chlorenchyma and inner epidermis. The outer integument degenerates totally during grain development with the inner integument becoming an acellular layer and forming the seed coat (testa). Compounds found in the testa determine the seed coat colour (Section 2.5.1). Below the testa is a cuticular layer and the hyaline layer consisting of crushed nucellar cells which borders the aleurone layer (Figure 2.4). The pericarp and testa are normally non-living at grain maturity.

2.2.2.5 Environmental effects on grain development

The time course of grain development, from anthesis to maturity, will be affected by the prevailing environmental conditions the plant experiences. The principal environmental factor governing grain development is temperature (Macleod and Duffus, 1988; Wardlaw, 1994), with high temperatures generally leading to more rapid grain development. Thermal timescales are often used to allow accurate modelling of plant growth and development, and they can similarly be applied to grain development (Weir *et al.*, 1984; Tottman *et al.*, 1985). Other factors such as water supply (Brooks *et al.*, 1982; Barlow *et al.*, 1980), humidity (Tashiro and Wardlaw, 1990) and light intensity (Wardlaw, 1994) have relatively minor effects on grain development after anthesis. A study in which some of the grains were removed from the ear at anthesis, generated greater numbers of endosperm and aleurone cells in the remaining developing grains causing them to be larger than normal (Radley, 1978).This illustrates that any

environmental factor adversely affecting grain fertilisation is likely to exert some influence on the subsequent development of the remaining grains. Our understanding of the effects of the environment on the physiological mechanisms governing grain development is relatively poor, with the effects of temperature only really investigated.

Short periods of high temperatures (21/16 to 36/31 °C for 2 days) close to anthesis can cause various morphological abnormalities in grains (Tashiro and Wardlaw, 1994). A high temperature at this stage in grain development may be having a direct effect on the cell division process, or it may be exerting an indirect effect on the levels of plant growth regulators controlling cell division. Although high temperature increases cell division there was no indication of greater cell numbers in the endosperm of barley grains grown at a range of temperatures from 15-30 °C (Macleod and Duffus, 1988). This suggests that the faster rate of cell division at high temperatures is counteracted by a shorter duration of the cell division phase.

In the grain filling period high temperatures tend to reduce starch accumulation and the duration of grain growth, and hasten the onset of senescence, leading to lower mature grain weights. At this stage in development high temperature appears to be affecting the enzymic pathways involved in the conversion of assimilate (sucrose) to starch storage reserves, predominantly by inhibiting the activity of the enzyme starch synthase (MacLeod and Duffus, 1988). Temperature has also been shown to effect the levels of plant growth regulators such as gibberellic acid (GA) in the developing grain (Radley, 1976), as well as the sensitivity of tissues in the grain, to these plant growth regulators (Norman *et al.*, 1982). It is thus apparent that a series of complex interactions governs the response of the developing grain to temperature.

2.3. The formation of high *alpha*-amylase activity in wheat.

The mechanisms leading to *alpha*-amylase production during grain development and maturation have been extensively studied revealing a complex procedure which is not yet fully understood (Hill and MacGregor, 1988; Kermode, 1990; Jones and Jacobsen, 1991). There are four physiological mechanisms which may lead to high *alpha*-amylase activity in harvested wheat grain (Kettlewell, 1989):-

(1) Retention of pericarp *alpha*-amylase in the grain until harvest (RPAA).

(2) Pre-maturity *alpha*-amylase activity in the absence of sprouting (PMAA).

(3) Pre-maturity sprouting before the onset of dormancy (PrMS).

(4) Post-maturity sprouting after dormancy break (PoMS).

Pre-harvest sprouting (PHS) damage is the term used to define the germination of grain in the ears of the parent plant before it can be harvested. PHS reduces the yield and quality of the crop eventually harvested. Yield can be reduced by an estimated 10% due to the loss of sprouted and depleted grains during the threshing process (Belderok, 1968). Additionally, the specific weight of the harvested crop will be decreased due to hydrolysis of storage reserves in the grain endosperm. The initiation of *alpha*-amylase synthesis in the grain is caused by a complex series of interactions between environmental conditions, plant genotype and the biochemical events controlling grain development and maturation.

2.3.1 The *alpha*-amylase isoenzymes

Early electrophoretic studies revealed the existence of two groups of *alpha*-amylase in wheat grain with differing isoelectric points (pI). These were classified on the basis of their appearance during grain development (Olered and Jonsson, 1970). "Green" amylase isoenzymes were found in immature grain (watery ripe to soft dough stage) and had a pI between 4.5-4.8 (low pI). They have also subsequently been identified as

occurring in germinating grains (Gale and Ainsworth, 1984). "Malt" amylase isoenzymes were only identified in germinating grains and had a pI between 6.0-6.5 (high pI).They have also been identified in grains during the latter stages of grain maturation (soft dough to caryopsis hard stage (Flintham and Gale, 1988). These enzymes have subsequently been referred to by many terms (Table 2.4). Additionally, a third group of *alpha*-amylases has been identified. These have been termed α-AMY-3 (Baulcombe *et al.*, 1987) and have a very high pI of about 10 (Daussant and Renard, 1987). These isoenzymes were identified in the outer pericarp early in development but subsequently rapidly declined. In the interest of clarity, the nomenclature α-AMY-1 and α-AMY-2 as used by Hart and Gale (1986) will be used to distinguish between the *alpha*-amylase isoenzymes in this review.

Table 2.4 : Various terms used to define two groups of *alpha*-amylase isoenzymes.

Alpha-amylase group	*Alpha*-amylase group	Reference
High pI	Low pI	Olered and Jonsson, 1970.
Malt	Green	Olered and Jonsson, 1970.
Group I	Group II	Sargeant and Walker, 1978.
G III	G I	Marchylo *et al.*, 1980.
Group 6	Group 7	Nishikawa *et al.*, 1981.
α-AMY-1	α-AMY-2	Hart and Gale, 1986.

The *alpha*-amylase isoenzymes follow defined developmental patterns during grain development (Marchylo *et al.*, 1980), with the differing isoenzymes responsible for the differing causes of high *alpha*-amylase activity in harvested wheat grain (See Sections 2.4-2.7).

2.3.2 The physiology of *alpha*-amylase production in wheat

In the aleurone layer of mature cereal grains *de novo* synthesis of *alpha*-amylase

is induced by gibberellic acid (GA) (Paleg, 1960 ; Briggs, 1963). The immature aleurone layer of wheat is however normally unresponsive to exogenously applied GA_3.

Experiments using de-embryonated grains have shown that GA_3 responsiveness of the aleurone layer can be induced/increased by subjecting it to a period of drying (Nicholls, 1979; King and Gale, 1980), a high temperature treatment (27 °C for 8 hours) (Norman *et al.*, 1982) or a low temperature (5 °C for 20 hours) treatment (Singh and Paleg, 1984) or by incubation in a simple buffered medium (2 mM Na-acetate buffer, pH 5.5, 20 mM calcium chloride and 15 µM chloramphenicol for 72 hours) (Cornford *et al.*, 1986). Furthermore, simply detaching immature grains from the ear has been shown to induce GA_3 sensitivity in the aleurone layer (Nicholls, 1986). The effectiveness of these treatments in inducing *alpha*-amylase synthesis was dependent on the developmental age of the grain. It is, therefore, clear that the aleurone layer has the intrinsic ability to synthesize *alpha*-amylase before the grain reaches harvest maturity. Additionally, developing grains of wheat contain sufficient concentrations of gibberellins to induce *alpha*-amylase production by responsive aleurone tissue (Radley, 1976). It is thus clear that during grain development, regulatory control mechanisms must be in operation which prevent high *alpha*-amylase activity developing.

During specific stages of grain development it is therefore possible that these control mechanisms are disrupted by certain environmental conditions leading to high PMAA (Gale *et al.*, 1983). The responsiveness of the aleurone layer to GA is thought to develop naturally during grain drying after the grain has reached its maximum fresh weight (Mitchell *et al.*, 1980a). The rate of grain drying of de-embryonated immature grain has been shown to affects its responsiveness to exogenously applied GA_3, with rapidly dried grains having reduced ability to respond to GA_3 (Nicholls, 1979). However, it has also

been shown that drying *per se* is not essential for the development of GA_3 sensitivity (Nicholls, 1986). This is supported by observations that wheat may sprout in the ear if it matures in continuously wet conditions (PrMS) (King, 1993). A discussion on the four mechanisms of *alpha*-amylase formation now follows.

2.4 Retention of pericarp *alpha*-amylase activity (RPAA)

The α-AMY-2 isoenzymes produced early in development are located in the pericarp of the grain. Their role is believed to be the degradation of starch granules present in the parenchyma cells of the maternally derived pericarp tissue. The α-AMY-2 activity in the grain then usually declines to undetectable levels as terminal differentiation of the pericarp occurs (generally after the grain loses its green colouration).

Rapid drying or premature shrivelling, may result in the formation of dry immature grains, with measurable levels of α-AMY-2 retained in the pericarp at harvest. This is possibly the reason why the HFN of grain often rises once grain is harvested, as the activity of α-AMY-2 in the pericarp of such grains declines (Olered and Jonsson, 1970; Morgan *et al.*, 1986). It has also been suggested that premature shrivelling and retention of active α-AMY-2 may have caused the lower HFN noted in some take-all (*Gaeumannomyces graminis* var. *tritici*) infected plots (Bateman *et al.*, 1990). In Canadian growing conditions, frost damage during grain maturation has been connected with the retention of α-AMY-2 activity in harvested grain (Kruger, 1989). This source of *alpha*-amylase activity is, however, unlikely to be widespread under UK conditions.

Synthesis of the same α-AMY-2 isoenzymes also occurs in germinating grains, appearing 2-3 days after α-AMY-1 isoenzymes (Gale and Ainsworth, 1984). The activity of α-AMY-2 isoenzymes present in the harvested grain is however only of minor significance compared to the activity of α-AMY-1 isoenzymes present. It has been

estimated that only 16% of the total *alpha*-amylase activity present in germinated wheat is due to α-AMY-2 isoenzymes (Sargeant and Walker, 1978 ; Marchylo *et al.*, 1987).

2.5 Post-maturity sprouting in wheat (PoMS)

PoMS is a widespread problem occurring in many wheat growing regions of the world (Derera, 1990). Wheat crops grown in the UK (Flintham and Gale, 1988), France (Couvreur *et al.*, 1994), Germany (Belderok, 1968), Scandinavia (Lallukka, 1976), Chile, Argentina, Brazil (Linhares and Dotto, 1980), South Africa (Marais and Kruis, 1983), the Northwestern states of the USA (Briggle, 1980), Western Canada (LaCroix *et al.*, 1976), China (Feng and Wenyan, 1993), Australia (Mares, 1993) and New Zealand (Humphrey-Taylor and Larsen, 1990) are all at risk from a high incidence of PoMS in years when periods of wet weather prevent the harvest of mature grain. PoMS caused by dormancy break, is an intermittent problem in the UK varying in severity from year to year, due to its dependence on environmental conditions (Greer, 1950 ; Farrand, 1972 ; Flintham and Gale, 1988). In the UK in 1977, two weeks of wet weather in mid-August delayed the harvesting of already ripe grain (Hough, 1990). This led to a high incidence of PoMS (4.5% of grains) and meant the UK average HFN only reached 127 (Anon, 1977). Delayed harvesting increases the likelihood that the grains natural protection against germination, dormancy, will be broken. Dormancy conferred a selective advantage on seeds as it allowed dissemination in time and/or space from their parent plant, avoiding unfavourable conditions for germination and ensured the survival of the "fittest" grains. In wheat this allowed seeds to survive in hot dry environments until conditions favourable for germination occurred. Selection procedures used by man in the last 150 years to select high yielding cultivars, have also unwittingly selected for reduced dormancy in cultivars. Early plant breeders grew two generations of a cultivar a year by using diverse growing

Plate 2.1 : Post-maturity sprouting in a wheat ear in the field.

environments. This sped up the selection process but also selected against cultivars with long dormancy periods. This has led to the problems of PHS and associated high *alpha*-amylase activity in grain.

Once dormancy is broken, the occurrence of sufficient rainfall will stimulate the germination of grains in the wheat ear (Plate 2.1)(Belderok, 1961 ; Belderok, 1968). The basic process of germination can be simplified into the following five stages :-

1) Grain imbibes water.

2) Gibberellic acid (GA) secreted by the scutellum diffuses to the aleurone layer.

3) Signal transduction initiates *alpha*-amylase synthesis in the GA-sensitive aleurone layer.

4) *Alpha*-amylase is secreted into the grain endosperm and amylolytic hydrolysis of starch granules begins.

5) Sugars produced by hydrolysis of storage reserves are absorbed by the scutellum and utilised as raw materials by the embryo for growth and development.

The wheat embryo has also been shown to account for a small amount of *alpha*-amylase first detected in the grain during germination (Mares, 1987a). Whether this is simply due to contamination of embryo tissue with aleurone tissue is unclear, particularly as the complete removal of the aleurone tissue from the embryo is a difficult operation. The amount of *alpha*-amylase produced by the embryo/scutellum is however relatively limited in comparison with the much larger aleurone tissue which is quantitatively the most important source of *alpha*-amylase activity during germination (Mares, 1987a).

2.5.1 Effects of genotype on grain dormancy

The genotype of wheat has a very significant effect on its dormancy. It has long been known that red-grained cultivars possess greater dormancy than white-grained cultivars (Hutchinson *et al.*, 1948; Miyamoto and Everson, 1958). This difference in testa pigmentation is attributable to the presence of *R* (red) / *r* (white) alleles located on chromosomes 3A (*R2*), 3B (*R3*) and 3D (*R1*) (Metzger and Sibaugh, 1970). White-grained cultivars possess the three recessive alleles *r1r2r3* and do not produce the red phlobaphene pigment. The possession of a dominant *R* allele is usually associated with increased sprouting resistance and increasing the *R* gene dosage from *R1r2r3* to *R1R2r3* to *R1R2R3* has been shown to additively increase sprouting resistance (Flintham and Gale, 1990). The *R* gene effect is thought to be pleiotropic with the phlobaphene or its catechin/catechin tannin precursors acting as inhibitors to germination (Gale, 1989).

The *R* alleles are however not always effective in conferring sprouting resistance. This is demonstrated by the fact that several cultivars possessing the genotype *R1R2R3*, including the UK cultivar Haven, have short periods of dormancy and are very susceptible to sprouting (Flintham, personal communication). The fact that some cultivars possessing the same *R* allele genotype have differing dormancy duration suggests that other genotypic factors are responsible for determining dormancy duration , with the *R/r* alleles genetically linked to grain dormancy, but acting as a marker gene (Gale, 1989). Support for this is provided by the fact that white-grained (*r1r2r3*) cultivars which contain no *R* alleles have varying degrees of dormancy duration (Mares, 1987b). Additionally the cultivar Kenya 321 (*r1r2r3*) is reported to possess two recessive genes which enhance its dormancy (Bhatt and Derera, 1980).

The effect of genotype on dormancy duration is strongly modified by interactions with the environment. This strong environmental interaction has hindered the elucidation of the dormancy period that particular field-grown cultivars are likely to experience from year to year.

2.5.2 The effects of environment on grain dormancy

It has long been reported that warm, dry weather during grain development will result in reduced dormancy compared to development in cool, wet weather. Systematic studies in controlled environments on the effect of weather conditions during grain development revealed that the dormancy duration of a particular cultivar of wheat was dependent on the accumulated daily mean temperatures above 12.5 °C reached during the dough stage (Belderok, 1961; Belderok, 1965).

i.e. Σ [(Max Temperature °C + Minimum Temperature °C)/2] - 12.5 °C

Large accumulated temperatures during this period led to a shorter period of

dormancy than smaller accumulated temperatures. This relationship has been used as the basis of several sprouting warning systems, with varying degrees of success (Section 2.10). An investigation of meteorological conditions during defined stages of grain development in several cultivars grown in the field for 11 to 20 years in Norway, also revealed that higher temperatures and more intense global radiation tend to decrease seed dormancy. This was particularly apparent in the 10 day period before the grains reached yellow ripeness (38% moisture content). Higher rainfall, higher air humidity and a high rainfall / temperature ratio were also shown to increase the duration of dormancy (Strand, 1989). The level of correlation between these environmental variables and dormancy duration did however vary between cultivars. It was also noted that meteorological conditions only began affecting seed dormancy during a period approximately 20 days before yellow ripeness.

The extent of dormancy induced in the grain is also believed to be influenced by the rate (King and Gale, 1980) and timing of desiccation of the grain (King, 1993), which will be affected by temperature conditions. Additionally, high temperatures after harvest have been shown to cause reduced dormancy duration (Hagemann and Ciha, 1987).

Monitoring the expression of dormancy is complicated by the temperature dependence of dormancy expression, with low temperatures (10 °C) inducing earlier germination than higher temperatures (20 °C and 30 °C), in grains exposed to the same environmental conditions (George, 1967). Generally the range of temperature over which seeds will germinate and the maximum germination achieved increases with increasing seed age (Gosling *et al.*, 1981). Loss of dormancy is, therefore, manifested by an increase in the range of conditions that grains will germinate in. Any studies investigating the effect of environmental conditions on dormancy duration must therefore also consider the crucial

role of the germination temperatures employed in the investigation if differences in dormancy are to be identified. Until it is clear which specific environmental factors are important in determining grain dormancy, the amount of sprouting in commercial field crops will remain difficult to predict, due to year to year differences in weather conditions.

2.6 Pre-maturity sprouting (PrMS)

In contrast to PoMS relatively little is known about the phenomenon of pre-maturity sprouting (PrMS). In 1977, a considerable degree of PrMS was observed in grains at or prior to the dough-ripe stage corresponding to 45% moisture content (Mitchell *et al.*, 1980b). PrMS was thought to be a contributory factor in causing the low average (175 s) HFN of the UK wheat crop in 1987 (Flintham and Gale, 1988; Hough, 1990). This phenomenon was not however reported on a widespread scale (Gold, 1991), and it therefore seems likely that PMAA and PoMS were the most significant processes affecting the HFN of grain in that year (Morgan, 1988).

Similarly, observations from field experiments in 1987 revealed the ability of grain to sprout pre-maturely in the ear, following the onset of ripening but prior to the onset of dormancy (Flintham and Gale, 1988; Flintham, 1990). PrMS was seen in the cultivars Bersée and Maris Huntsman during the sixth week after anthesis, when the grain moisture content fell from 48-44% (Flintham, 1990). Comparing these two cultivars it appears that PrMS is independent of PMAA , as Bersée demonstrated higher levels of PrMS compared to Maris Huntsman, which had higher levels of PMAA. In this respect PrMS is similar to PoMS, in that both appear to be independent of PMAA (Gale *et al.*, 1987). PrMS has also been observed in the field in other countries. An extremely cool ripening period was believed to be the cause of sprouting before yellow ripening in some cultivars in Finland in 1974 (Lalluka, 1976). Similarly studies on Australian cultivars have shown that in ear

germination can occur by about 50 days post anthesis in non-desiccated grain (King, 1993). Interestingly, the years of occurrence of PrMS in the UK have coincided with years of a high incidence of wheat orange blossom midge (WOBM) (*Sitodiplosis mosellana,* Géhin) infection (Oakley, 1993). It is thus possible that PrMS may have been induced by a combination of WOBM damage and weather conditions, as opposed to being a direct effect caused by the weather (Plate 2.2). Observations on 1977 sprouted grains, showed that many of the grains demonstrated the growth of only the coleoptile, which failed to penetrate the seed coat, which is symptomatic of WOBM damage (Mitchell, 1980). Similarly the occurrence of PrMS in Finland (Lalluka, 1976) may be due to WOBM damage as the pest is widespread in that country (Helenius and Kurppa, 1989).

Plate 2.2 : Pre-maturity sprouting caused by wheat orange blossom midge (WOBM) (*Sitodiplosis mosellana,* Géhin) damage.

2.7 Pre-maturity *alpha*-amylase activity (PMAA)

Some British cultivars of winter wheat have long been known to produce high *alpha*-amylase activity in ungerminated grain (Stewart, 1964; Bingham and Whitmore, 1966). The amount of this activity is suggested to be exacerbated by slow grain drying conditions before harvest (Gale *et al.*, 1983). Any environmental factor which adversely affects the control mechanisms regulating *alpha*-amylase production in developing grains, may therefore be responsible for the generation of high PMAA. PMAA has also been identified as a widespread potential problem in many other wheat growing regions of the world (Table 2.5), with many cultivars capable of producing high PMAA under certain environmental conditions. PMAA may however be a potentially larger problem than so far envisaged. Many breeder's screens measure *alpha*-amylase activity or HFN of the grain at harvest. This masks any effect of PMAA as any such *alpha*-amylase is coupled with post dormancy break sources of the enzyme (Gale, 1989). PMAA is thus often overlooked and may only become apparent in poor crop ripening conditions. The isoenzyme responsible for high PMAA is thought to be α-AMY-1. This isoenzyme was identified by isoelectric focusing, as the main constituent of the high PMAA in Maris Huntsman, appearing a week before harvest ripeness (Gale *et al.*, 1983). Previously this enzyme had been shown to be present in unsprouted grains of the cultivar Champlein (Sargeant, 1980) and also in several Canadian cultivars before they reached grain maturity (Marchylo *et al.*, 1980).

Using a combined histochemical and blotting procedure Cornford *et al.*, (1987a) demonstrated that in Fenman, α-AMY-1 activity was found in the ventral portion of the grain in association with the aleurone tissue. In contrast, α-AMY-1 activity in Maris Huntsman was identified after 40 DAA in the embryo, but was not seen until 48 DAA in

Table 2.5 : Examples of cultivar variation in pre-maturity *alpha*-amylase activity and the relative effect of the environment.

Cultivar	Origin	Relative amount of *alpha*-amylase activity	Relative effect of environment on *alpha*-amylase activity	Reference Source
Professeur Marchal	Belgium	High	Not documented	Bingham and Whitmore, 1966
Maris Nimrod	UK	Medium	High	Pushman and Bingham, 1976
Champlein	France	Medium	Not documented	Sargeant *et al.*, 1980
Cypress	Canada	High	Not documented	Marchylo *et al.*, 1980
Bezostaya I	Russia	Medium	Medium	Gale *et al.*, 1983
Snabbe	Sweden	Low	High	Gale *et al.*, 1983
Mardler	UK	High	Low	Seymour, 1984
Fenman	UK	High	Low	Cornford and Black., 1985.
Norman	UK	High	Medium	Cornford and Black., 1985
Maris Huntsman	UK	Medium	High	Gale *et al.*, 1987
Lerma 52	Mexico	High	Low	Mares and Gale, 1990
Spica	Australia	High	Low	Mares and Gale, 1990
BD 159	Australia	High	High	Mares *et al.*, 1994

the endosperm (Gale *et al.*, 1987). From this evidence it was suggested that either α-AMY-1 was generated in the embryo region and diffused into the endosperm, or that a stimulus from the embryo caused high α-AMY-1 activity in the scutellar or aleurone tissues near the embryo initially. This evidence suggests that the embryo may play a causal role in generating high PMAA.

Studies on the regulation of *alpha*-amylase activity in the developing embryo of the cultivar Sappo, which is not susceptible to the formation of high PMAA, (Cornford and Black, 1985) have revealed that embryos in developing wheat grains do not normally contain any detectable *alpha*-amylase activity (Cornford *et al.*, 1987b). However, isoelectric focusing of extracts from excised embryos incubated in a buffer medium for 48 hours revealed that α-AMY-2 isoenzymes were produced by 15-40 DAA embryos, whereas α-AMY-1 isoenzymes were only produced by 25-35 DAA embryos (Garcia-Maya *et al.*, 1990). This illustrates that the embryo has the capacity to produce *alpha*-amylase, but in normal development this capacity is suppressed. Suspected factors which may cause this *in situ* suppression of *alpha*-amylase activity were further investigated in this study. It was, noted that abscisic acid (ABA) was not detectable in incubated excised 30 DAA embryos which produced α-AMY-1, suggesting this may be the factor preventing α-AMY-1 formation in the embryo. Adding ABA to 30 DAA excised embryos, completely suppressed the appearance of α-AMY-1 isoenzymes and inhibited the activity of α-AMY-2 isoenzymes by approximately 40%. Similar effects were seen when 30 DAA excised embryos were incubated in 0.6 M mannitol, which has a similar osmolarity to the endosperm. Using cDNA probes of α-AMY-1 revealed that low osmotic potential and ABA can both prevent accumulation of α-AMY-1 mRNA transcripts, while GA increases their amount. This suggests that embryonic α-AMY-1 genes are suppressed *in situ* by

endogenous ABA and/or by the osmotic environment and in some respect by low levels of endogenous GA (Garcia-Maya *et al.*, 1990). Any adverse effects on the regulatory mechanism governing *alpha*-amylase activity in the embryo tissue may, therefore, lead to the generation of high PMAA. Factors which can affect the magnitude of PMAA in wheat are now discussed.

2.7.1 Effects of genotype on PMAA

The genetic basis of the wheat grain has posed specific problems when analysing grain characteristics relating to *alpha*-amylase activity. The chromosome complement (ploidy) of various tissues in the grain differs (See Section 2.5.2.1), with *alpha*-amylase activity stimulated by both the scutellum (embryo-tissue, diploid) and the aleurone layer (triploid), studying the inheritance of specific genes regulating *alpha*-amylase activity and sprouting in wheat has been problematic (Gale, 1989). An early study on fourteen wheat cultivars clearly illustrated large cultivar differences in the level of *alpha*-amylase present in grain before harvest in the absence of sprouting (Bingham and Whitmore, 1966). Professeur Marchal was the cultivar demonstrating the highest PMAA. This cultivar's inclusion in many UK plant breeding programmes was believed to be the source of PMAA problems in certain specific wheat cultivars. It was, however, noted that is unrelated to the susceptibility of the grain to sprout in the ear. A study on four winter wheat genotypes (Maris Huntsman, Bezostaya, Bersée and Koga II) however noted, that once dormancy was complete, the cultivars with high PMAA, Maris Huntsman and Bezostaya, showed more rapid development of the sprouted phenotype than cultivars with low PMAA, Bersée and Koga II (Gale *et al.*, 1983).

A study in which single chromosomes from the cultivar Bezostaya I (high PMAA) were substituted into the cultivar Hobbit'S' demonstrated that there was no correlation

between the chromosomal effects on PMAA and conventional sprouting (Flintham, 1990). Substitution of the chromosomes 4D and 7A increased PMAA in the substituted line, illustrating that these chromosomes were the most significant in determining the relatively low levels of PMAA in Hobbit'S'. This substitution did not have any significant effect on PoMS. These results add support to the hypothesis that PMAA is distinct from conventional sprouting. The relationship between PMAA and PrMS was less clear, with substitutions of the 4D and 7A chromosomes also significantly increasing PrMS of the substituted line.

Very few studies have been undertaken to identify the genes causing high PMAA. A comparison of 29 F_4 lines from the cross Professeur Marchal (high PMAA activity) x Nord Desprez (low PMAA activity) revealed a discontinuous distribution of 23 low and 6 high *alpha*-amylase lines (Bingham and Whitmore, 1966). This suggests that PMAA is controlled by a single or at most two duplicate recessive genes (Gale, 1976). Further evidence that the susceptibility of cultivars to high PMAA is controlled by one or two recessive genes is provided by monosomic lines of an Australian cultivar (Spica) which were analysed for *alpha*-amylase activity (Mares and Gale, 1990). The majority of the monosomic lines had high *alpha*-amylase activity. In contrast, monosomic 6B plants could be separated into groups with high and low *alpha*-amylase activity in an approximate 3:1 ratio. Chromosome counts revealed that the high *alpha*-amylase lines were disomic and the low *alpha*-amylase lines were monosomic. This suggests that *alpha*-amylase activity in Spica is mediated by a single recessive gene possibly located on chromosome 6B. This finding is under going further investigation in an effort to identify whether this gene is present in UK cultivars (Mares and Gale, 1990).The limited evidence available therefore suggests that genetic control of PMAA is simple, being mediated by one or two recessive

genes.

The fact that susceptibility to high PMAA seems to have a simple genetic basis, implies that it should be possible to eliminate this gene successfully from wheat breeding programmes (Flintham and Gale, 1990). As the presence of the trait in many cultivars suggests that PMAA may be linked to, or pleiotropic with an important agronomic gene (Mares and Gale, 1990), selection of low PMAA lines in breeding programmes may be problematic due to adverse effects on other agronomic traits. The dependence of expression of the trait on the environment has also hindered selection of low PMAA lines, as the precise environmental stimulus for high PMAA is not known. Recently in Australia, several potential new cultivars had to be withdrawn from breeding programs prior to, or following release, due to the identification of their susceptibility to PMAA (Mares and Mrva, 1993). The identification of such problems late on in the breeding program is extremely costly both in terms of time and resources.

2.7.2 Effect of gibberellic acid insensitivity (*Rht*) genes on PMAA

Gibberellic acid (GA) insensitive dwarf (*Rht*) genes (Gale and Youssefian, 1985), carried on group 4 chromosomes have been widely exploited in wheat breeding programs, producing commercial cultivars with short, stiff straw, and associated lodging resistance. Dominant *Rht* alleles cause an insensitive seedling growth response to applied GA relative to tall recessive *rht* genotypes. These genes have also been shown to affect PMAA in grain.

A study using near isogenic lines of Maris Huntsman demonstrated that the dwarfing alleles, *Rht1* and *Rht3*, and the tall allele, *rht* had differential effects on PMAA in grain (Gale *et al.*, 1987). In the *rht* lines, 51% of grains expressed PMAA, however in the *Rht1* and *Rht3* lines this was reduced to 30% and 8% of grains respectively. The

PMAA in those grains expressing it was also found to be lower in the *Rht1* and *Rht3* lines. In a similar study using near-isogenic lines of Maris Huntsman (*Rht1*,*Rht2*,*Rht1+2*, *Rht3*), a reduction in PMAA at harvest of 54%, 61%, 51% and 73% respectively, was seen compared to *rht* lines (Gold, 1991). *Rht* alleles can often mask the effect of genes which lead to high PMAA. Analysis of 100 F_3 lines derived from a cross between the Australian semi-dwarf (*Rht1*) cultivar Suneca, whose parentage includes the high *alpha*-amylase cultivar Spica and Ford (a low *alpha*-amylase cultivar) produced several lines with a HFN less than either parent and 9 lines with a HFN similar to Spica. This suggests that Suneca had inherited a high *alpha*-amylase gene from Spica, but that its effects were overcome by the *Rht1* allele (Mares and Gale, 1990). The effects of *Rht* alleles are small when compared to cultivar differences. For example, the *Rht2* cultivars Fenman and Longbow had approximately four times the level of *alpha*-amylase activity, compared to an isogenic *Rht2* line of Maris Huntsman (Flintham, 1990). This finding illustrates that other genes causing high PMAA can be epistatic to the effects of *Rht* alleles. This limits their usefulness in overcoming PMAA in plant breeding programmes. The *Rht3* allele may be most useful in F_1 hybrid *Rht3/rht3* wheats (Gale and Youssefian, 1985). Allele dosage effects in the triploid endosperm would mean that 50% of the F_2 grains (those whose genotype was *Rht3/Rht3/Rht3* or *Rht3/Rht3/rht3*) could be expected to have some genetic protection from PMAA. This effect would however only reduce *alpha*-amylase levels by about 40% thus also limiting their usefulness (Flintham and Gale, 1982).

How the *Rht* alleles affect GA sensitivity and PMAA is not known. Isogenic lines of Norin 10 (*Rht1*) and Tom Thumb (*Rht3*) have been found to contain high levels of endogenous GA_1, the typical gibberellin in vegetative tissues (Lenton *et al.*, 1987). However, no difference in the level of the principal gibberellin in developing grain (GA_{54})

was identified (Gaskin *et al.*, 1980). This suggests that the *Rht* genes may control different functions in vegetative tissue and in the developing grain. Production of an inhibitor to GA activity or more direct effects on a putative GA receptor have also been proposed as possible causes of GA insensitivity in *Rht* lines (Gale and Marshall, 1973). The fact that *Rht* alleles affect the sensitivity of tissue to GA and can also affect PMAA suggests that GA plays an important role in stimulating PMAA.

2.7.3 Effects of environment on PMAA.

In a comparison between environments the Australian variety Spica and the Mexican variety Lerma 52, both produced high *alpha*-amylase activity and a low HFN in both warm, dry and cool, wet conditions, whereas Maris Huntsman only produced a low HFN in cool, wet conditions (Mares and Gale, 1990). It was noted, however, that PMAA in Spica and Lerma 52 was markedly stimulated by the cool, wet conditions. This demonstrates that PMAA can be enhanced by environmental factors even in cultivars which constitutively seem to produce high PMAA (e.g. Fenman, Spica), whereas in other PMAA susceptible cultivars (e.g. Maris Hunstman, BD159) more precise environmental conditions are required for high PMAA to occur (See Table 2.5).

The significance of weather conditions on the PMAA in commercial crops was highlighted in 1985. Cool, wet weather led to delayed crop maturity and harvesting, but no signs of visible sprouting in the grain (Gale and Lenton, 1987). However, the mean HFN of the UK wheat crop only reached 161 s (Anon, 1986a). This was largely attributed to high PMAA activity (Gale *et al.*, 1987; Flintham and Gale, 1988), although it has been suggested that the widespread low HFN of UK wheat crops in that season may have been due to incipient sprouting (Gold, 1991). This effect reduced the usage of home grown wheat in British flour, resulting in the import of an extra 1.15 million tonnes of wheat, at

a cost of approximately £ 100 million (Gale and Lenton, 1987). High PMAA was also identified in Maris Huntsman in the UK in 1987 (Flintham and Gale, 1990), contributing to the low HFN of UK wheat crops in that year (Morgan, 1988). Other field studies in the years of 1984 (Cornford and Black, 1985) and 1990 (Gold, 1991) when dry weather led to an absence of PoMS and below average *alpha*-amylase activity, failed to detect any high PMAA in Maris Huntsman, although high PMAA was seen in the cultivar Fenman.

2.7.3.1 Grain drying-rate and PMAA activity

PMAA generally starts to rise during the grain drying period in certain susceptible cultivars. In a study of Maris Huntsman, *alpha*-amylase activity began to increase as the water content of grains fell from 40-20% (Gale *et al.*, 1983). Similarly, a rise in *alpha*-amylase activity in Mardler (Seymour, 1984) and Fenman (Cornford and Black, 1985) coincided with a decline in moisture content from 40-20 %.

Earlier studies on detached grains had shown that slow drying of immature grains (30 DAA) increased the potential of these grains to produce *alpha*-amylase in response to exogenous GA_3 (Nicholls, 1979; King and Gale , 1980). Small changes in slow drying-rates had large effects on the inducibility of *alpha*-amylase in response to exogenously applied GA_3. Different cultivars also showed large differences in their level of response to drying conditions and *alpha*-amylase inducibility.

The effect of drying-rate on PMAA in the grains on intact plants has been examined in controlled-environments and in the field, in an effort to determine whether a relationship between PMAA and grain drying-rate exists. By maintaining relative humidities of 56% and 92% in controlled-environment cabinets, "fast" (~1.85% moisture loss day^{-1}) and "slow" (~0.8% moisture loss day^{-1}) grain drying-rates respectively were generated by Gale *et al.*, 1983. The effect of these conditions on PMAA in the spring

wheat cultivars Snabbe and Aotea, was to significantly increase *alpha*-amylase activity in the grain during the drying period, with the "slow" grain drying treatment, enhancing PMAA compared to the fast drying treatment. This enhancement of *alpha*-amylase activity due to the slow drying of the grain between a moisture content of 40%-20% was also seen when PMAA was compared from Maris Huntsman grown under controlled environment conditions (approximately 2% moisture loss day^{-1}), and in the field in 1981 (approximately 3.3% moisture loss day^{-1}). Gale *et al.*(1983) suggested that any factor which prolonged the period of grain drying between 40-20% moisture could enhance PMAA in susceptible cultivars. This effect was most notably demonstrated in commercial field crops grown in 1985, where below average evaporative demand after 600 °C-days post-anthesis delayed crop ripening leading to low HFN (Hough, 1990).

Rather more tenuous support for the role of grain drying in determining PMAA comes from the analysis of grains in ears of tall (*rht*) Maris Huntsman (Gale *et al.*, 1987). This revealed that grains in the lower central region of the ear, particularly in floret 2, contained the highest amounts of PMAA, whereas grains from the extreme spikelets and distal floret positions within the spikelets showed lower PMAA. Gale *et al.* (1987) proposed that grains in the distal floret positions would be more exposed to favourable environmental drying conditions and would therefore be more likely to experience a faster drying-rate and concomitant lower PMAA. No further evidence has been found to substantiate this claim.

The relationship between grain drying-rate and *alpha*-amylase activity has been examined in an effort to try and predict the HFN of crops. No clear relationship between grain drying-rate and HFN at harvest was identified in commercial crops of Avalon and Mercia grown in 1988 (Kettlewell and Astbury, 1990). Perhaps this was not surprising as

the HFN of these crops was relatively high, covering a narrow range of low *alpha*-amylase activity, with the weather conditions ensuring that grain drying-rates were relatively high compared to those observed by Gale *et al.*, (1983). The effects of grain drying-rate on PMAA could therefore have been masked by other factors such as the starch properties of the grain, which influence the HFN more significantly when *alpha*-amylase activity is low (Ringlund, 1983). Subsequent continuing field studies in 1989 and 1990, did reveal an overall linear relationship between the reciprocal of *alpha*-amylase activity and grain drying-rate (Astbury and Kettlewell, 1991). This relationship was however poor, with wide seasonal differences and considerable variation occurring in any one year. This implies that some other seasonal factor apart from the grain drying-rate may be responsible for stimulating increased PMAA.

Furthermore, in another field experiment, an effort was made to manipulate differing grain drying-rates, using covering and wetting treatments, involving the use of polythene "tents" to cover plots of Avalon, Fenman, Apollo and Mission (Gold, 1991).This revealed that, within cultivars, drying-rate appeared to be unrelated to *alpha*-amylase activity, although Fenman did have a slower rate of drying and higher *alpha*-amylase content than the other cultivars. The warm and dry weather conditions in these years of study, again led to fast drying-rates and low *alpha*-amylase activity and which may have masked a relationship between grain drying-rate and *alpha*-amylase activity.

Gold (1991) used polyethylene glycol (PEG) to manipulate the grain drying-rate in detached cultured ears of near isogenic lines of Maris Huntsman, but did not identify any relationship between grain drying-rate and *alpha*-amylase activity. In this study the slowest grain drying-rate was 1.07% moisture loss day^{-1}, again suggesting that the relationship between *alpha*-amylase activity and grain drying-rate may only be apparent

at slower drying-rates. Gold *et al.* (1990) proposed that environmental factors may have a more pronounced effect on stimulating PMAA earlier in grain development, with grain drying-rate modulating the extent of this effect. A possible clue to the environmental "trigger" factor causing PMAA comes from observations taken from wheat plants grown in northern hemisphere glasshouses. High *alpha*-amylase activity was noted in the ears of Maris Huntsman grown in the glasshouse compared to field grown samples (Evers and Ferguson, 1980). Similarly high *alpha*-amylase activity was noted in Fenman when it was grown in the glasshouse (Gold, 1991). Earlier work in 1964 on the cultivar Professeur Marchal also hinted that glasshouse plants had higher PMAA (64.0 versus 44.3 Farrand units) than field plants (Bingham and Whitmore, 1966). Gold (1991) suggested that high humidity, high temperature or a large diurnal temperature range may be the "glasshouse" environmental factors responsible for stimulating high PMAA. Interestingly a similar effect has been found in comparison of wheat plants grown in the field and in southern hemisphere glasshouses (Mares and Mvra, 1993). It should be noted however that conditions were much cooler in southern hemisphere glasshouses (Max. 25 °C) compared to the plants grown in the field (Max. 38 °C). The glasshouse factors responsible for stimulating increased PMAA in cultivar BD-159 were therefore proposed as cool temperatures and high humidity (Mares *et al.*, 1994).

2.7.3.2 Temperature and PMAA

There is limited evidence that exposure to a period of high temperature during early development increases PMAA. Randall and Moss (1990) noted in studies on the effects of temperature on the grain quality of the Australian cv. Olympic, that a brief (3 day) exposure to high temperature (35/30 °C) early in grain development (20 DAA) significantly decreased the HFN of the flour at harvest. Whether this decrease in HFN was

actually due to PMAA as opposed to the other forms of *alpha*-amylase accumulation, or changes in starch properties is uncertain. In 1985 when PMAA was high in commercial crops in the UK, the temperatures during the first 600 °C-days of the grain growth were above average (Hough, 1990). This is again circumstantial evidence that high temperatures early in grain development may affect PMAA, as the low HFN of the UK crops in that year were largely attributed to PMAA (Morgan, 1988). Additionally unexpectedly high *alpha*-amylase activity is often seen in plants affected by temperature faults in plant growth rooms (Black, M., personal communication).

The mechanism by which high temperature could affect PMAA expression is unclear. Radley (1976) demonstrated that exposure of cv. Kolibri ears to high temperatures (20 °C and 25 °C), led to significantly higher levels of gibberellin in the grain 17 and 24 DAA, compared to ears treated at 15 °C. With gibberellin levels implicated in PMAA formation this finding also supports the suggestion that high temperatures may be responsible for triggering high PMAA.

Additionally, it has been shown that aleurone tissue from undried immature developing wheat grains can be made fully sensitive to gibberellic acid by incubation at 27 °C for at least 8 hours, followed by exposure to a lower temperature for just a few seconds. Norman *et al.* (1982) proposed that the high temperature treatment was altering the sensitivity of the aleurone cells by altering the homeoviscous properties of their membranes. Other studies have illustrated that transformation of aleurone cells to a GA-responsive state is temperature dependent. The effect of high temperature treatments on the GA-responsiveness of immature aleurone tissue was, however, shown to have only a marginal promotive effect (Cornford *et al.*, 1986). No clear reason for the discrepancy between these two results is apparent.

In contrast, cool temperatures have also been implicated as a possible stimulus for high PMAA. An experiment in which the cultivar BD-159 was transferred to a controlled environment cabinet (12-17 °C) for 7 days and then returned to a warm, dry environment to mature, revealed that transfer to cool temperatures 21 and 28 days after anthesis increased the mean *alpha*-amylase activity in the grain (Mrva and Mares, 1994). In field experiments in northern Japan it has also been noted that cool temperatures can increase grain *alpha*-amylase activity. In years when mean temperatures during grain ripening were low (approximately 15 °C), high PMAA was detected. In years when the mean temperature was higher (18-20 °C) , no high PMAA was noted (Mrva and Mares, 1994). It is however unclear whether the effect of the low temperature is modulating PMAA by effects on grain drying- rate or whether low temperature is actually stimulating expression of PMAA.

A mechanism by which cool temperatures could stimulate PMAA expression has not been elucidated. Low temperatures (5 °C) have been shown to induce GA_3-sensitivity in mature GA_3-insensitive (*Rht3*) aleurone tissue (Singh and Paleg, 1984). The ability of this stimulus to induce GA_3-sensitivity in immature aleurone tissue which has yet to attain GA_3-sensitivity is open to conjecture. Specific temperatures in the field may therefore cause changes in the sensitivity of the aleurone to gibberellic acid, and with sufficient moisture available for *alpha*-amylase synthesis, this may lead to increased PMAA (Gold, 1991).

From the above review it is clear that both genotype and environment are important factors in determining expression of PMAA, with climatic affects capable of amplifying genotypic effects. There also several agronomic factors which can affect *alpha*-amylase activity in grain although the significance of these effects is minor compared to

meteorological conditions (Kettlewell, 1993).

2.8 The effects of agronomic inputs and crop husbandry on *alpha*-amylase activity and Hagberg falling number

2.8.1 Effects of spring applied nitrogen fertiliser

Several studies have been made on this topic. Pushman and Bingham (1976) showed that there was a tendency for grain *alpha*-amylase activity to decrease with increasing nitrogen fertiliser application, although there were significant cultivar differences in the magnitude of this change. Gooding *et al.* (1986a) also demonstrated that the HFN of two cultivars of winter wheat (Avalon and Brimstone) increased linearly with nitrogen fertiliser application. This relationship was however, less pronounced in another cultivar (Mission). Further evidence that spring application of nitrogen fertiliser improves the HFN of grain in some seasons was provided by Macdonald and Vaidyanathan (1987). In 10 out of 13 trials (carried out in a range of soil types and climatic zones) an improvement in HFN was recorded with increasing nitrogen fertiliser application up to the optimum for yield. Confirmation that increasing levels of spring applied nitrogen were in fact reducing *alpha*-amylase activity, rather than altering starch properties (Ringlund, 1983) was provided by Astbury and Kettlewell (1990) who measured both *alpha*-amylase activity and HFN.

Other studies have found that increasing nitrogen fertiliser does not result in improvements in HFN (Hayward, 1987) or decreases in *alpha*-amylase activity (Astbury and Kettlewell, 1992). Possible reasons for this include lodging of the crop, which increases with increasing spring nitrogen fertiliser application and was prevalent in many of the experiments (See Section 3.4.) The prevalence of lodging cannot solely explain these observations and other unknown seasonal factors are thought to be involved in the

process.

Gooding *et al.* (1986a) suggested that increasing nitrogen fertiliser delays the crop from reaching maturity. Crops receiving little or no nitrogen fertiliser were thus thought likely to mature earlier, possibly experiencing *in situ* deterioration in HFN due to incipient sprouting. An alternative explanation is provided by a study by Kettlewell and Cooper (1993). Nitrogen fertiliser reduced *alpha*-amylase activity and also reduced grain moisture content by accelerating the grain drying-rate. This supports the hypothesis that there is a relationship between grain drying-rate and PMAA (Gale *et al*, 1983). Other seasonal factors are also likely to influence the drying-rate of maturing grain and the amount of PMAA observed. Thus the beneficial effect of nitrogen fertiliser application may be counteracted by these effects, leading to the observed variability in the trials (MacDonald and Vaidyanathan, 1987).

2.8.2 Effects of late-season foliar fungicides

The effects of late season foliar fungicides on grain *alpha*-amylase activity have not been directly studied, instead the HFN of the grain has been measured and inferences made as to the level of *alpha*-amylase activity present (Gooding *et al.*, 1987). These studies have revealed that fungicide application can reduce the HFN of grain (Gooding *et al.*, 1986b; Morgan *et al.*, 1986; Salmon and Cook, 1987; West, 1990). Other studies have found no significant effect of fungicides on HFN (Myram and Kelly, 1981; Clare *et al.*, 1990; Clark, 1993).

The effect of fungicides on the HFN of grain is principally thought to be mediated by effective disease control (Salmon and Cook, 1987; Cook and Hims, 1990). Effective disease control prevents early senescence of flag leaves due to fungal infection and maintains green leaf area duration (Gooding *et al.*, 1987). This maintenance of

photosynthetically active tissue is generally beneficial to yield (West, 1990), however this effect can be detrimental to HFN as it delays ripening of the grain. Gooding *et al.* (1987) showed that fungicide treated grain had a significantly higher moisture content than untreated grain, with a correspondingly significantly lower HFN at harvest. It was proposed that the delayed drying of the grain during grain development could have led to an increase in PMAA (Gale *et al.*, 1983), thus explaining the effect of the fungicide on the HFN. Alternatively it may be that delayed ripening of the crop pushes harvest back into wet weather periods where the observed fall in HFN may be due to PoMS (Stevens *et al.*, 1988; Clark, 1993).

The observation that fungicides can delay the senescence of leaves even when little disease is apparent (Dickinson and Walpole, 1975) has led to the suggestion that some fungicides have a more direct effect on plant physiology and *alpha*-amylase activity. Triazole fungicides such as propiconazole can affect gibberellic acid levels (Buchenauer, 1984) which are known to be involved in the regulation of *alpha*-amylase activity (Paleg, 1960). The results of an experiment in which fungicide treatment with the triazole, prochloraz, plus fenpropimorph had a more severe effect in decreasing HFN than treatment with chlorothalonil and fenpropimorph (at similar disease control levels) have been used to support the suggestion that some fungicides have direct effects on plant physiology and *alpha*-amylase activity (Stevens *et al.*, 1988). This is however rather anomalous as triazoles act as GA inhibitors and would therefore be expected to decrease *alpha*-amylase activity and increase HFN, not decrease HFN as observed. West (1990) noted that two applications of the triazole propiconazole plus fenpropimorph had no effect on HFN. The significance of direct effects of fungicides on plant physiology and *alpha*-amylase activity is therefore unclear. These effects are also interactively related to the

variable site and year factors which have a much greater effect on HFN. It is nonetheless clear that the principal effect of fungicides is to enhance green leaf area duration, thereby prolonging grain maturation. This may increase the risk of the crop encountering adverse environmental conditions which could lead to high *alpha*-amylase activity developing.

2.8.3 Effects of desiccants

In contrast to fungicides it has been proposed that preharvest desiccants may improve the drying rate of maturing grain (Anon, 1986b). This would reduce the risk of high PMAA forming (Gale *et al.*, 1983) and thus avoid grain developing a low HFN in poor crop ripening conditions.

Studies on the effect of the desiccant glyphosate on the HFN of grain have been inconclusive. This has been primarily due to the years of study coinciding with hot, dry weather conditions, unfavourable to the formation of PMAA. The application of a desiccant therefore had no observable beneficial effect on the high HFN of the grain (Stevens *et al.*, 1988; Astbury and Kettlewell, 1992). Additionally, the time of application was found to be problematical. To avoid any deleterious effects on yield glyphosate could only be applied at or below a grain moisture content of 30%. The effects of the desiccant during the earlier stages of grain drying, which maybe more critical in PMAA synthesis were therefore not studied (Gold *et al.*, 1990).

2.8.4 Effects of lodging

Lodging of crops has serious consequences on grain quality. Crops that are lodged invariably have much lower HFN than unlodged crops. The main reason for this is the increased humidity and availability of water to the grain in lodged crops. Imbibition of water is therefore possible, and grains will begin to sprout once dormancy has been broken. Early drilling of wheat can result in more profuse tillering and canopy

development and enhances the risk of lodging occurring during heavy rainstorms in the summer. Similarly, the application of high levels of nitrogen fertiliser in the spring encourages excessive canopy development and can cause lodging problems in wet summers. The use of plant growth regulators has been shown to have a beneficial effect on HFN, by reducing the occurrence of lodging in the crop (Stevens *et al.*, 1988).

2.8.5 Effects of cultivar contamination

Contamination arising from cereal volunteers in the field or carelessness in harvesting and storing crops of wheat can have significant effects on the HFN of the harvested crop. Vaidyanathan (1987) showed that a single grain of Fenman in nineteen grains of Avalon was capable of reducing the pure HFN of Avalon from 440 s to 250 s. Similarly it was shown that contamination of a sample of Minaret with 1.5 % of Fenman reduced the HFN of Minaret from 275 s to 244 s (Morgan *et al.*, 1988). It is therefore essential that good field husbandry, careful harvesting and planning of the storage of grain are undertaken to overcome the adverse effects of this factor.

2.8.6 Effects of post-harvest gravity table separation

By fractionating wheat samples according to differences in grain density, gravity table separation can be used to improve HFN. Germinated grains can be removed from samples as they have a lower density than ungerminated grains. Gravity table separation also removes weed seeds and other contaminants which can lower HFN (Garstang, 1993). The improvement in the HFN of samples using gravity table separation does not, however, occur in all batches of low HFN wheat and is not always related to an increase in bread-making quality (Hook *et al.*, 1988).

2.9 Potential solutions to the high *alpha*-amylase activity and sprouting problem

Several lines of research are being undertaken in an effort to find solutions to the

problem of high *alpha*-amylase activity in wheat. These can be summarised as :-

1) Elimination of sprout-susceptible lines in breeding and selection programs (Derera, 1989b; Flintham, 1993).

2) Detection of sprouted crops (Jensen *et al.*, 1984).

3) Separation of sound and sprouted grains (Tkachuk *et al.*, 1990 ; Bettge and Pomeranz, 1993).

4) Inhibition of germination enzymes using metabolic inhibitors (Zawistowska *et al.*, 1988; Henry *et al.*, 1993).

5) Remedial actions in milling and baking technologies (Henry *et al.*, 1987; Strandberg *et al.*, 1988; Kent and Evers, 1994).

6) Prediction of potential risk of sprouting by forecast, allowing accelerated harvest to avoid potential deterioration in crop quality (Belderok, 1968; Karvonen and Peltonen, 1991).

Of these potential solutions to the problem, pre-harvest prediction of sprouting risk and HFN may be the most useful and cost-effective for growers. The cost benefits of using a predictive model are likely to outweigh the costs of using subsequent post-harvest remedial action to improve crop quality, which are of generally limited effectiveness anyway. A prediction scheme would be useful even with further improvements in the sprouting resistance of cultivars as environmental weather conditions will still exert a considerable effect on the sprouting susceptibility and *alpha*-amylase levels in cultivars.

The establishment of a prediction scheme will not be a simple task. It has been stated that "the hazard of sprouting in Britain will continue to be the least predictable and most difficult factor to overcome in the breeding and reliable production of wheat for bread making" (Bingham *et al.*, 1991). Nonetheless several attempts at prediction

schemes have been made by researchers in other countries.

2.10 Sprouting and Hagberg falling number prediction schemes

Prediction schemes based on Belderok's relationship between accumulated temperature ($\sum$[(Max. Temperature °C +Min. Temperature° C)/2]-12.5) during the dough stage and the duration of dormancy (Belderok, 1965) were used in Netherlands and in the Schleswig-Holstein region of Germany in the 1960s (Belderok, 1968). These relied on many regional observers recording the beginning and end of the dough stage of the principal wheat cultivars and the daily maximum and minimum temperature. This data was used to compile reports on the risk of sprouting, which were then disseminated to growers via advisers, radio reports and telephone. This scheme was reportedly in successful operation for at least four years.

In France an agro-climatological model based on accumulated temperature during the dough stage and the genetic resistance level of the cultivar to sprouting has been developed (Belderok, 1973; Corbineau *et al.*, 1993). The genetic resistance of a cultivar to sprouting is classified into one of five groups (very sensitive, sensitive, some resistance, resistant, very resistant) with resistant cultivars requiring higher accumulated temperatures than the more sensitive cultivars to bring about the same attenuation in dormancy. If dormancy duration is predicted to be below 10 days, then the cultivar is considered to be at risk from sprouting and warnings are issued in growers' bulletins.

Other researchers have failed to identify a satisfactory relationship between the simple Belderok temperature-sum rule and the duration of dormancy and thus there is doubt as to its usefulness as a prediction system warning of sprouting. In Germany, no relationship was seen between the Belderok temperature-sum rule and the duration of dormancy (Schrodter and Grahl, 1974). It was proposed that the simple Belderok rule

only applied to relatively low temperatures, with higher temperatures inducing a longer dormant period than would be expected using the Belderok system. It was thus suggested that the relationship between accumulated temperature during the dough stage and the length of dormancy was more an optimal curve. Schrodter and Grahl (1974) concluded that the length of the dormancy period was dependant on evapotranspiration, solar radiation and temperature during the 7 days preceding harvest ripeness. On this basis a sprout warning service was in operation in most parts of West Germany in the late 1970's, although its successfulness is not documented (Belderok and Habekotté, 1980). Variations from Belderok's rule were also identified in Sweden. Olsson and Mattson (1976) warned that accumulated temperature could not be used as the sole basis of an efficient warning system in Swedish conditions. Similarly, it has been shown that Belderok temperature-sum rule did not result in any higher correlation with grain dormancy than with the mean temperature during the same period (Strand, 1989).

One of the problems with Belderoks' temperature-sum rule is the imprecisely defined nature of the start and end of the dough stage (Strand, 1989). A difference of one or two days in defining these time points could lead to wide differences in accumulated temperatures. This would be particularly apparent if maximum and minimum temperatures were high. This difference could account for some of the variation and discrepancies found using Belderoks' scheme. A moisture content of 45-22% has also been used as a basis to define the duration of the dough stage (Mitchell *et al.*, 1980b). Measuring moisture content gives a more quantitative and less error prone indicator of grain developmental stage. Its use in the studies discussed above would have enabled more accurate examination of the Belderok temperature-sum rule to be made.

It has also been shown that pre-maturity sprouting can occur in some cultivars

before the dough stage is reached, thus negating the usefulness of Belderoks' prediction scheme (Lallukka, 1976; Mitchell *et al.*, 1980b). However, this pre-maturity sprouting may be attributable to WOBM damage rather than meterological conditions (Oakley, 1993)(Section 2.4).

In Sweden a series of pre-harvest HFN samples are taken to inform farmers of the trend in HFN of the crop before harvest (Larsson, 1987). In Finland the National Agricultural Extension Service has developed a "falling number service" to inform farmers when the sprouting of wheat grains is most probable during harvest. This is a very simplistic system and is limited by the sparse number of localities where ear samples are collected and a time delay of about two days between sampling and issuing of results (Karvonen *et al.*, 1991).To try and overcome these problems multiple regression analysis has been used to determine inter-relationships between climatic factors and sprouting in wheat (Karvonen *et al.*, 1991). Climatic factors incorporated into multiple regression statistical analysis were mean and maximum daily temperature, daily precipitation, daily relative humidity and daily global radiation. The basic weakness of this approach in predicting HFN is the fact that these functions do not reflect the physiological mechanisms that are the actual cause of variations in HFN. Further studies have led to the development of a dynamic explanatory type model for HFN of wheat (Karvonen and Peltonen, 1991) and rye (Laurila *et al.*, 1992). Testing this model using data from two spring wheat cultivars grown in 1989 and 1990 revealed that the predictions were very poor with wide "forks" i.e. the predicted HFN was approximately ± 100 s (Teittinen *et al.*, 1994). It was suggested that including factors such as humidity and wind, and relating these and other meteorological factors to changes in grain moisture, may improve the accuracy of the prediction model.

In the UK preliminary investigations have been undertaken examining the relationships between pre-harvest HFN samples and combine harvest HFN. Samples taken at less than 30% moisture content, at 15 and 19 ADAS Experimental Husbandry farm sites in 1986 and 1987 respectively, showed good correlation (Stevens *et al.*, 1988). Similarly, experiments on field crops of Avalon in 1988 and 1990 identified a close relationship between pre-harvest (x) and combine-harvest (y) HFN ($y = 1.10\,x$-27, $R^2 = 0.8$, $P < 0.001$). This relationship was however weaker with the cultivar Mercia ($y = 0.72\,x + 111$, $R^2 = 0.3$, $P < 0.001$) (Kettlewell, 1993).

None of the above mentioned schemes and models considers the development of PMAA. To produce an accurate prediction scheme in the UK requires a precise understanding of the effects of the environment on PMAA as well as the other routes causing high *alpha*-amylase activity in the grain (Kettlewell, 1993). By gaining an understanding of these relationships it should be possible to develop a model predicting the occurrence of high PMAA. By incorporating this model with other models on *alpha*-amylase formation, an overall scheme could thus be devised allowing accurate pre-harvest prediction of *alpha*-amylase activity, Hagberg Falling Number (HFN) and risk of sprouting in wheat. Identifying the environmental factors stimulating PMAA would also be beneficial to breeders as it may enable them to establish screening methods to unequivocally identify PMAA susceptible cultivars and remove them from breeding programs. The principal aim of this thesis was therefore to investigate the effect of environmental variables on PMAA and try and predict its occurrence in the field.

3. Field Experiments Investigating the Effects of Environmental Factors on Pre-Maturity *Alpha*-Amylase Activity (PMAA).

3.1. Introduction

The exact environmental conditions which stimulate high PMAA in the grain before harvest are at present unclear. This is predominantly due to the erratic occurrence of the phenomenon, which has precluded detailed observation of the event in the field and has made investigation into PMAA problematical (Section 2.7). The limited current knowledge of the precise environmental factors stimulating PMAA, also means that it is not possible at present, to establish conditions in controlled-environment facilities which will guarantee the occurrence of high PMAA. Field experiments relying on inherent environmental conditions have thus formed a large part of this and other research work investigating the problem of high PMAA. This does have certain advantages as previously stated by Gold (1991). Large uniform populations of plants are required in experiments investigating PMAA, which can be accommodated more easily in the field, than in the more space limiting conditions in controlled-environment facilities. Large populations of plants are required to avoid sampling error, and account for the large variation in PMAA between and within wheat ears (Evers and Ferguson, 1980). Additionally, time course studies monitoring enzyme activity through grain development generally require a large number of plants, as destructive sampling is used. Furthermore, the general desire to include several cultivars differing in sensitivity to PMAA can be accommodated more easily in field experiments.

The main collaborative project established field experimental sites at four locations between the years 1994 and 1996, giving ten site x year combinations. It was anticipated that a wide range of environmental conditions would be experienced at each field

experimental site over the years of the study given their differing location and long term mean weather conditions (Table 3.10). This was anticipated to cause a wide variation in the routes causing high *alpha*-amylase activity in the grain, thus enabling the degree of susceptibility of particular cultivars to high PMAA to be identified.

Once samples with low HFN (high *alpha*-amylase activity) were identified in field experiments it was essential to discriminate between high *alpha*-amylase activity due to PMAA, as opposed to the other distinct routes which led to high *alpha*-amylase activity in the grain (Section 2.3.1). The relative occurrence of the various routes causing high *alpha*-amylase activity under UK field conditions could, thus, be ascertained. To facilitate this a range of techniques was developed to discriminate between the four different routes causing high *alpha*-amylase activity in harvested grain.

3.1.2 Identification of four routes leading to high *alpha*-amylase activity in the grain

3.1.2.1 Visual inspection

A basic visual inspection of grains gave an initial indication of the source of *alpha*-amylase in the grain. The presence of green immature grains in a sample gave an indication that RPAA may be present. Similarly a rupture in the pericarp covering the embryo or a protuberance of a radicle or plumule was a clear indication that germination (PoMS or PrMS) had commenced. Characteristic damage to grains caused by orange wheat blossom midge larvae was also identifiable (Oakley, 1993), suggesting that PrMS may have occurred (Plate 2.2). The absence of any visible signs of sprouting and a low HFN suggest PMAA may be present in the sample.

3.1.2.2 Fluorescein dibutyrate (FDB) staining

This technique allows the visualisation of hydrolase activity in germinated kernels using the lipase-sensitive fluorochrome, fluorescein dibutyrate (Jensen and Heltved,

1982). In the presence of lipase activity in germinating grains this substrate is broken down to produce yellow fluorescent fluorescein.

Lipase activity in germinating grain

↓

Fluorescein dibutyrate → Butyrate + Fluorescein
(non-fluorescent) (fluorescent)

Lipase activity in germinating wheat embryos appears within 12 hours of imbibition (Heltved, 1984), whereas visual signs of sprouting are often not visible until 48 hours of imbibition (Jensen *et al.*, 1984). This technique thus allows sprouting not visible to the naked eye to be identified in the grain. This qualitative assay uses lipase activity as a separate indicator of germination activity to *alpha*-amylase activity. PMAA does not breakdown FDB, as no lipase activity is associated with this route of formation and so no fluorescence is visible. Several studies have investigated the relationships between visual sprouting, FDB staining and *alpha*-amylase activity in an effort to correlate quantitative relationships between them (Munck, 1987; de Francisco *et al.*,1989). The fact that PMAA is not detected by FDB staining adds support to the suggestion that FDB should be used solely as a qualitative indicator of sprouting and not quantitatively related to *alpha*-amylase activity (Henry and McLean, 1986).

3.1.2.3 Iso-electric focusing (IEF)

IEF was used to identify the presence of α-AMY-1 and/or α-AMY-2 isoenzymes in flour samples. The isoenzyme profiles obtained were then compared to characteristic profiles produced at different stages during grain development and germination to give an indication of the cause of high *alpha*-amylase activity in the grain (Sargeant and Walker, 1978 ; Gale and Ainsworth, 1984). The fact that during early PoMS only α-AMY-1 isoenzymes are synthesised (Cejudo, *et al.*, 1995), with α-AMY-2 isoenzymes being

synthesised 48 to 72 hours later (Gale and Ainsworth, 1984), prevents the separation of early PoMS and PMAA using this technique. PMAA is usually caused solely by α-AMY-1 isoenzymes (Section 2.7), thus early PoMS may be erroneously be attributed to PMAA.

3.1.2.4 *Beta*-limit dextrin gel and iodine staining test and Phadebas gel assay

Beta-limit dextrin (Cornford *et al.*, 1987b) and Phadebas (Seymour, 1984 ; Evers *et al.*, 1995) gel assays were used to indirectly locate the region of *alpha*-amylase activity in transverse and longitudinal sections of grains. The *beta*-limit dextrin gel substrate is broken down following exposure to *alpha*-amylase activity in the sectioned grains into smaller chain carbohydrates and maltose and glucose. Subsequent staining of the *beta*-limit dextrin gel with iodine allows the *alpha*-amylase activity in the grain imprint on the gel to be visualised. Unstained patches on the gel indicate where the *beta*-limit dextrin has been broken down into maltose and glucose by *alpha*-amylase activity, as these compounds do not form blue/black coloured polyiodide complexes. This allows *alpha*-amylase activity to be assigned to the embryo, the crease region, or the pericarp region of the grain (Cornford *et al.*, 1987b). In the Phadebas gel assay no staining is required as *alpha*-amylase activity causes the release of a Cibachron Blue dye from the dye-labelled starch substrate (Barnes and Blakeney, 1974) leaving clear patches on the grain imprint in the gel where *alpha*-amylase activity is present.

Combining the results from this series of tests allows the route of high *alpha*-amylase activity in the grain to be identified and attributed to the cause of a low HFN in the harvested grain (Table 3.1).

3.1.3 Experimental objectives

The effect of grain drying-rate on PMAA in grains on intact plants has been examined in controlled-environments and in the field, in an effort to determine whether

Table 3.1. Tests and results used to identify four routes causing high *alpha*-amylase activity in harvested grain.

Test	Identification of cause of high *alpha*-amylase activity in the grain			
	RPAA	PMAA	PrMS	PoMS
Visual sprouting	No (Green grains in sample)	No	Yes (Characteristic WOBM damage visible)	Yes
FDB staining	No	No	Yes (embryo region)	Yes (embryo region)
IEF	α-AMY-2	α-AMY-1	α-AMY-1 + α-AMY-2	α-AMY-1 + α-AMY-2
Beta-limit dextrin gel assay and/or Phadebas gel assay	Halo effect formed on gel (no localisation of activity around embryo)	Activity in crease region of grain only	High activity localised in embryo end of grain and along crease region after prolonged sprouting	High activity localised in embryo end of grain and along crease region after prolonged sprouting

a relationship between PMAA, HFN and grain drying-rate exists. The results from these experiments have not been conclusive but suggest that slow grain drying-rates enhance PMAA and decrease HFN (Section 2.7.3.1).

By monitoring the moisture content, *alpha*-amylase activity and Hagberg falling number of several cultivars, over ten site x year combinations it was anticipated that a broad range of grain drying-rates would be obtained in the field allowing a better analysis of the hypothesis that there is a relationship between *alpha*-amylase activity, Hagberg falling number and grain drying-rate between 40-20 % grain moisture content. These results would also allow the time of initial increase in PMAA to be more precisely defined. This is an important consideration if pre-harvest HFN samples are to be taken for predicting combine-harvest HFN (Kettlewell, 1993). Two studies have shown a relationship between pre-harvest HFN samples and combine-harvest HFN (Section 2.10). Taking a progressive series of pre-harvest samples gives a clear indication in the trend in HFN, but is costly in terms of both time and labour. Additionally the results produced only give growers a limited warning of the likely HFN of the crop as time of processing samples and distributing results can take 4-5 days. Establishing an effective single pre-harvest HFN sampling time point early in the development of the crop would enable an indication of the likely HFN of the crop to be established. This is, however, problematical due to the variation in *alpha*-amylase activity at particular time points in grain development, as similarly seen in triticale (Mares and Oettler, 1991).

Early pre-harvest sampling for HFN at the end of grain filling is hindered by native pericarp (α-AMY-2) amylase which may not have all been degraded by this stage in development (Section 2.4). Pre-harvest sampling must also be late enough in development for the initiation of PrMS and PMAA to be identified (Section 2.5, 2.6). It, however,

must also be sufficiently early for pre-harvest HFN prediction to be of practical use to the grower. The effects of PoMS on the relationship between pre-harvest HFN and combine-harvest HFN cannot be accounted for by early sampling as its effects occur later in the development of the grain (Section 2.7). The purpose of these experiments was to establish if a pre-harvest HFN sample could be used to predict the combine-harvest HFN in UK summer weather conditions, in the absence of PoMS. Coupling these results with an assessment of the risk of sprouting would then enable a prediction of combine-harvest HFN to be made (Kettlewell *et al.*, 1996).

In addition, four ancillary objectives relating to pre-harvest HFN sampling were defined and investigated.

a) The relationship between HFN from pre-harvest samples taken by hand and those taken by combine-harvesting was examined to see if sampling by hand gave an accurate representation of sampling by combine-harvesting. This was done as it was thought mechanical combining may generate different grain samples than from those taken by hand cutting samples (Bloom, 1985), *i.e.* small grains may be separated out by sieves and lost from combine sample (Hall, 1991).

b) Sample timing was also investigated to try and establish the earliest possible sampling point for pre-harvest HFN, and to see how the accuracy of the relationship between pre-harvest HFN and combine HFN varied over time.

c) The precision of pre-harvest sampling by hand for HFN was also investigated. This was undertaken to establish the number of the hand-samples needed to be taken, to enable a precise HFN value to be obtained.

d) Centralised analysis of pre-harvest HFN samples at HA was also compared to analysis of samples at their site of origin. This was undertaken to establish whether

centralised analysis of pre-harvest HFN samples was more preferable and feasible than analysis at dispersed sites.

To summarise, the main objectives of the field experimental work can be listed as:-

1) Identify the route causing high *alpha*-amylase activity in harvested grain.

2) Establish the degree of susceptibility of particular cultivars to high PMAA.

3) Examine the effect of natural variation in grain drying-rate between ten site x year combinations on PMAA and HFN.

4) Establish the time when PMAA first becomes detectable in the grain.

5) Establish if a pre-harvest HFN sample can be used to accurately predict combine-harvest HFN in UK summer weather conditions, in the absence of PoMS.

6) Test the practicality of the group experimental protocol and establish its suitability for use in an HFN prediction scheme (Section 1) in subsequent years.

It was also hoped comparisons between field experiments could be made, enabling the effects of specific environmental factors on high PMAA to be identified. These factors could then be examined in more detail and quantified using controlled-environment cabinets (Section 4).

3.2 Materials and methods

3.2.1 Site location

Details of the ten site x year combinations in the field experiments are listed in Table 3.2.

3.2.1.1 Harper Adams University College (HA)

Field experiments were undertaken at Harper Adams University College (52° 46'

N, 02° 23' W) in the years of 1993/4, 1994/5 and 1995/6. The field experiments were drilled using a 10 row Hege plot drill, at a seed rate of 375 seeds m^{-2}, with a row spacing of 12 cm and a plot length of 10 m. Double plots were drilled in 1994/5 and 1995/6.

3.2.1.2 Sutton Bonington (SB)

Field experiments were undertaken at University of Nottingham, Sutton Bonington, Leicestershire (52° 49' N, 01° 15' W) in the years of 1993/4, 1994/5 and 1995/6.The experiments were drilled using a Oyjord drill, at a seed rate of 375 seeds m^{-2}, with a row spacing of 13.2 cm and a plot length of 10 m.

3.2.1.3 ADAS Bridgets (AB)

Field experiments were undertaken at ADAS Bridgets, Martyr Worthy, Hampshire (51° 05' N, 01° 16' W) in the years of 1994/5 and 1995/6. The experiments were drilled using a Oyjord drill at a seed rate of 450 seeds m^{-2}, with a row spacing 12.5 cm and a plot length of 24 m.

3.2.1.4 University of Aberdeen (UA)

Field experiments were undertaken at Tillycorthie Farm, University of Aberdeen, Scotland (57 ° 18' N, 02°09' W) in the years of 1994/5 and 1995/6. The experiments were drilled using a Oyjord drill, at a seed rate of 375 seeds m^{-2}, with a row spacing of 13.2 cm and a plot length of 20 m.

3.2.2 Experimental design

The experiments consisted of eight cultivars of winter wheat (*Triticum aestivum*) including four UK cultivars (Haven, Hornet, Pastiche and Riband) and four French cultivars (Recital, Scipion, Soissons and Thesee) (Table 3.3). The UK cultivars were chosen on the basis of differences in their National Institute of Agricultural Botany (NIAB) ratings for Hagberg Falling Number (HFN) and sprouting resistance (Anon.,

Table 3.2 : Location of ten site x year combinations used in field experiments in 1993/4, 1994/5 and 1995/6.

Year	1993/4		1994/5				1995/6			
Site	HA	SB	HA	SB	AB	UA	HA	SB	AB	UA
Field	Near Cot Leasow	2	Swans Leasow	9	Nevada	Botany	Birds Nest	24	Arizona	Skillydams
Soil type	Very slightly stony sandy clay loam Clifton series	Sandy clay loam over Kueper Marl	Slightly stony sandy clay loam Arrow series	Sandy clay loam over Kueper Marl	Silty clay loam Free Draining	Freely drained sandy loam Tarves series	Slightly stony sandy clay loam Arrow series	Sandy clay loam over Kueper Marl	Silty clay loam Well Drained	Freely drained sandy loam Tarves series
Previous Crop	Grass	Winter Oats	Sugar Beet	Winter Oats	Oil Seed Rape	Oil Seed Rape	Potatoes	Winter Oats	Oil Seed Rape	Oil Seed Rape
Sowing Date	02 Nov	23 Oct	27 Nov	19 Oct	24 Oct	24 Oct	08 Nov	10 Oct	17 Oct	18 Oct
Harvest Date	19 Aug	29 Aug	(Fr) 02 Aug (UK) 10 Aug	14 Aug	04 Aug	21 Sep	02 Sept	02 Sept	04 Sept	18 Sept

HA - Harper Adams University College, Newport, Shropshire. **SB** - University of Nottingham, Sutton Bonington, Loughborough, Leicestershire.
AB - ADAS Bridgets, Martyr Worthy, Hampshire. **UA** - University of Aberdeen, Scotland.

Table 3.3 : HFN and sprouting resistance ratings of the UK and French cultivars grown in the field experiments.

Cultivar	Country of Origin	HFN Rating	Parentage	Sprouting Resistance Rating
Haven[1]	UK	C[5] (Medium)	(Hedgehog x Norman) x Moulin	2[5] (Low)
Hornet[1]	UK	D[5] (Low)	Norman x Hedgehog	7[5] (High)
Pastiche[1]	UK	A[5] (Very High)	Jena x Norman	7[5] (Very High)
Riband[1]	UK	C[5] (Medium)	Norman x (Maris Huntsman x TW161)	7[5] (High)
Recital[2]	France	High[6]	9369 x 267	2[6] (Very Low)
Scipion[3]	France	Very High[6]	?	8[6] (High)
Soissons[4]	France	Very High[6]	Jena x HN35	7[6] (High)
Thesee[3]	France	High[6]	?	3[6] (Low)

[1]Plant Breeding International, Trumpington, Cambridge, UK.
[2]ETS C.C. Benoiste, Ferme de Moyencourt, 78910-Orgerus, France.
[3]Verneuil, France.
[4]Florimond Desprez, BP 41-59242 Cappelle en Pévèle, France.
[5]NIAB ratings (Anon., 1991),
[6]ITCF ratings (Anon., 1995).

1991). Each UK cultivar possessed the PMAA susceptible cultivar Professeur Marchal in its lineage (Jarman *et al.*, 1993). The French cultivars were similarly selected on the basis of their sprouting rating (Anon., 1995). The experiments were designed as randomised split-plot experiments, consisting of three replicate blocks, with the main plots consisting of misted and non-misted plots and the sub-plots being the eight cultivars. The designs for the field experiments at HA are illustrated in Appendix 1. In 1994/5 and 1995/6 the French and UK cultivars were separated, to allow differential irrigation and harvesting of the plots. This was done as the French cultivars matured approximately 5-7 days before the UK cultivars in 1993/4. Additionally the plot size was doubled to two 2 x 10 m plots to allow more frequent and larger samples to be taken. The data presented in this thesis focuses solely on the results from the non-irrigated plots of UK cultivars. The results from the irrigated plots relate principally to PoMS and are presented elsewhere (Lunn *et al.*, 1998: Lunn *et al.*, 1999).

3.2.3 Crop Husbandry

Plots were subjected to standard agronomic practice except that the fungicide input was high to minimise the prevalence of ear diseases. Crop husbandry details for the experimental sites are provided in Appendix 3.

At HA in 1993/4 polypropylene netting with a mesh size of 153 mm, (Netlon®-Plant Support, LBS Group, Lancashire, UK) supported by posts, was placed tautly over the misted plots in Block 2, at a height of 0.5 m, on the 31 st May, 1994 (ZGS 37-55). This was to act as an insurance measure against lodging occurring later in the season. Lodging did not occur in any of the plots in the experiment. This netting was not used in subsequent years.

A Seedmaster Universal plot combine (F.Walter and H.Wintersteiger KG, Maschinenbau Riedinnkr, Austria) was used to combine plots at HA, whereas, a Sampo 2025 plot combine was used at AB, SB and UA.

3.2.4 Sampling methods

The experimental sampling protocol employed at all sites was based on a preliminary protocol devised by Dr. Gavin Lunn, Division of Agriculture and Horticulture, University of Nottingham. A sampling timescale based on accumulated temperature above a base temperature of 0 °C after ear emergence (ZGS 55), was used to determine sampling time points.

i.e. $\sum$ [(T °C daily maximum+T °C daily minimum)/2] = °C-days.

This was to try and ensure the comparability of data between sites and years, as temperature affects plant development rates. At 400 °C-days after ear emergence (approximately 300 °C-days after anthesis), sampling for all the main analyses commenced at 100 °C-day intervals until after harvest maturity.

Sampling points in the plots were systematically defined to minimise any effect on crop microclimate caused by removing ears. Plots were sampled from alternate ends in each of the three blocks of non-irrigated plots. The spacial layout for sampling the plots is illustrated in Figure 3.1.

3.2.5 Weather data recording

Daily weather observations were obtained from local weather stations located in the vicinity of field experiment sites. At HA in 1993/4 this was 600 m from field experiment site. At HA in 1994/5 and 1995/6 weather observations were obtained from a Hardi Metpole® (Hardi International A/S, Helgeshøj Allé 38, 2630 Taastrup, Denmark) located adjacent to the experiment (Plate 3.1). Additionally, a rain gauge (Rain-O-Matic®, ELE International, Hemel Hempstead, UK) was used to measure rainfall. A 2 kΩ thermistor probe (ST1), connected to a temperature integrator board in a Type MV2 microvolt integrator (Delta-T Devices Ltd, Burwell, Cambridge, UK) was also used to measure temperature in the field as a check.

Figure 3.1 : Spatial distribution of sampling points in field experiment plots at HA in 1993/4.

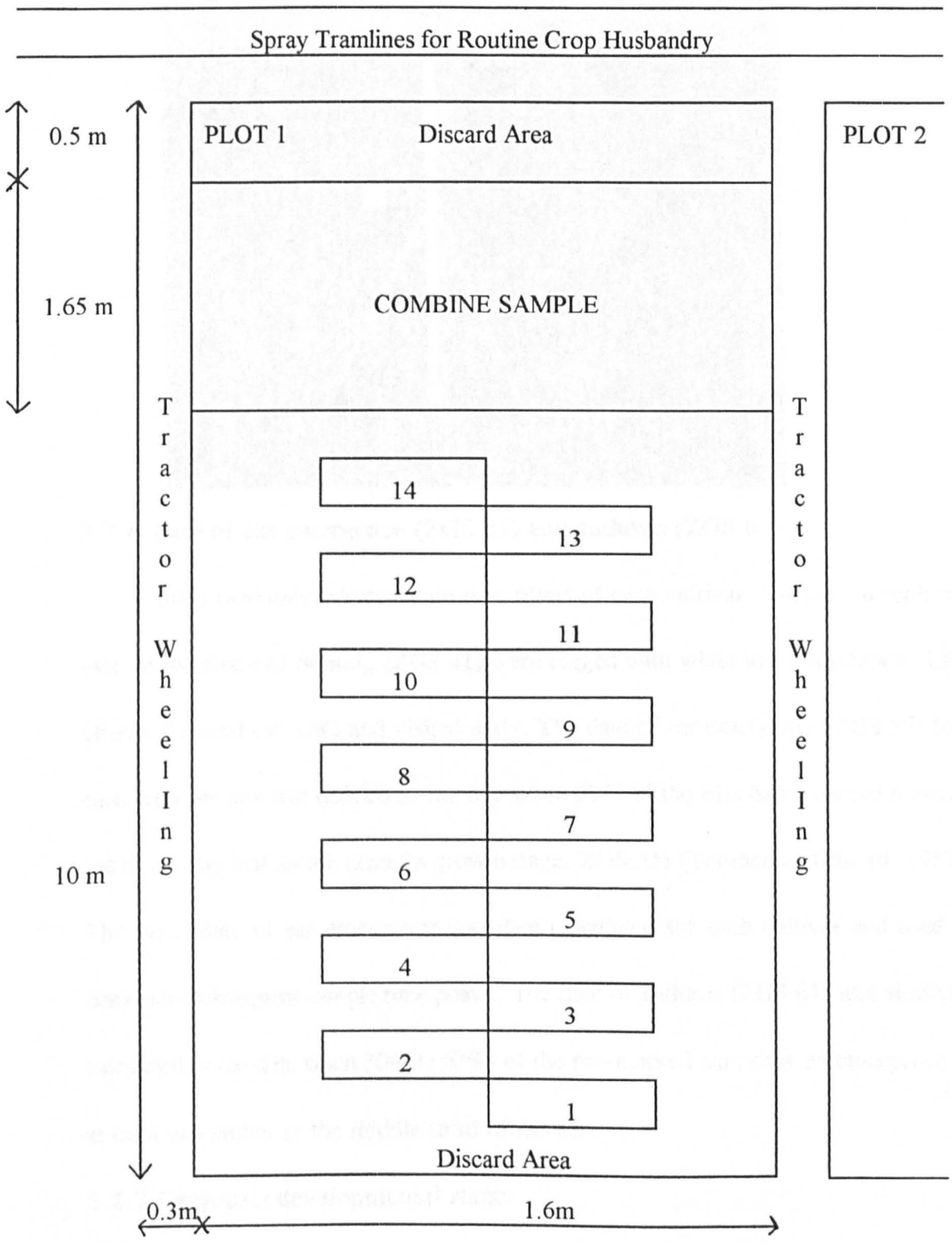
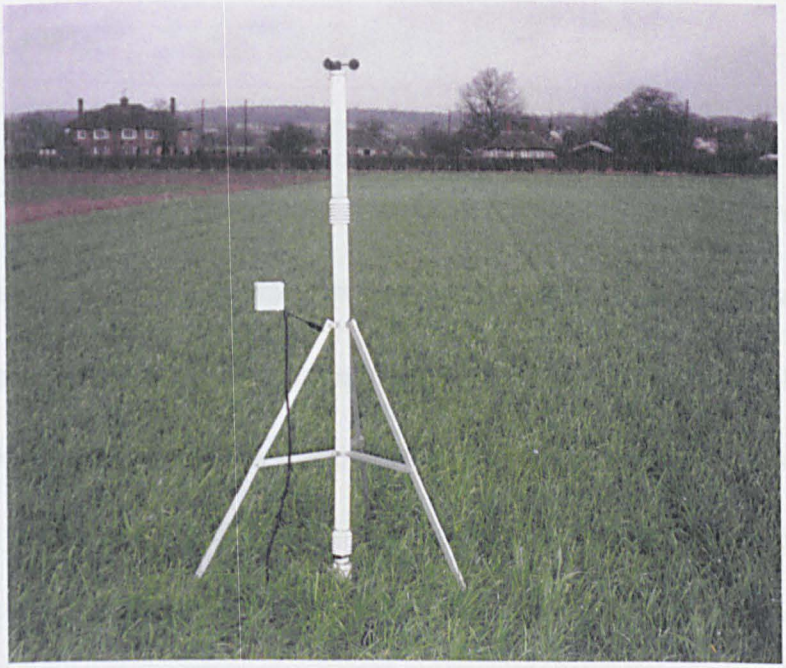


Plate 3.1 : Hardi Metpole used at HA to record weather data.



3.2.6 Date of ear emergence (ZGS 55) and anthesis (ZGS 61)

Sixty randomly selected main stem tillers of each cultivar, ten in each replicate plot, at the phase of booting (ZGS 41) were tagged with white lock-loop labels (LBS Group, Lancashire, UK) and visited daily. The date of ear emergence (ZGS 55) for each replicate plot was defined as the day when 50% of the ears had emerged halfway out of the flag leaf sheath (Zadoks growth stage, ZGS 55) (Tottman and Broad, 1987). The mean date of ear emergence was then calculated for each cultivar and used to determine subsequent sample time points. The date of anthesis (ZGS 61) was similarly calculated as the date when 30/60 (50%) of the pre-marked ears showed emergence of at least one anther in the middle third of the ear.

3.2.7 Caryopsis developmental stage

This was determined in three ways by reference to : accumulated temperature above 0 °C, percentage water content/dry weight and subjective assessment of milk,

dough and physiological maturity stages of randomly chosen caryopses (Tottman and Broad, 1987).

3.2.8 Moisture content and dry weight

Twenty ears from each replicate plot (x3) were removed at 100 °C-day intervals, wrapped in clingfilm, packed in an insulated box cooled with ice packs, and transferred to the laboratory for rapid analysis. Grains from florets 1 and 2 of the three central spikelets were pooled and their fresh weight recorded. These grains were then frozen and freeze dried at -60°C for 72 hours (Edwards 4k Modulyo Freeze Drier, Edwards High Vacuum, Crawley, West Sussex) to a constant mass. The % moisture content was then calculated:-

$$\text{Moisture Content (\%)} = \frac{\text{Fresh Weight-Dry Weight}}{\text{Fresh Weight}} \times 100$$

Additionally oven drying at 80 °C for 48 hours was used to rapidly determine the moisture content of the grain during the course of the experiment. In 1994/5 and 1995/6 rapid oven drying at 130 °C for 2 hours was used to determine moisture content of the grain.

Linear regression analysis was performed on site x year combinations where sufficient moisture content measurements were undertaken to allow analysis of the relationship between time and grain moisture content between 50-20 %. The grain drying-rate was equivalent to the absolute value of the slope of the lines obtained. The sampling timescale of °C-days after ear emergence (thermal time) was converted to days after ear emergence (time) to allow a grain drying-rate of % moisture loss day $^{-1}$ to be calculated. Using a thermal timescale of °C-days after ear emergence, would have led to grain drying-rates being quoted in % moisture loss °C-days $^{-1}$. These grain drying-rates

would have been similar for each site x year combination as the effects of temperature are accounted for in the thermal timescale.

Further linear regression analysis ($y = a + bx$) was then undertaken to examine the relationship between grain drying rate (x) and combine-harvest HFN (y).

3.2.8.1 Comparison of freeze-drying and oven-drying treatments

A comparison between oven drying at 80 °C for 48 hours and freeze drying was made on samples taken from HA in 1994 to examine if there was any significant differences in the moisture content values obtained by the two methods.

3.2.9 *Alpha*-amylase activity

The *alpha*-amylase activity in flour was measured using an air segmented flow autoanalyser (Skalar (UK) Ltd , York, UK.). This system is based on a spectrophotometer system described by Smith (1970), which utilises the Farrand technique (Farrand, 1964) to measure *alpha*-amylase activity in a sample extract.

3.2.9.1 Extraction of *alpha*-amylase from flour

Grain samples were ground in a hammer mill (Falling Number AB, Stockholm, Sweden) and 20 ml of extracting solution, 5 g l^{-1} sodium chloride and 0.2 g l^{-1} calcium chloride, (Barnes and Blakeney, 1974) was then added to two 1 g duplicate flour samples taken from the well mixed ground sample. The samples were then shaken for 5 minutes on an orbital shaker (Gallenkamp, Leicester, UK) at 100 r min^{-1}. These samples were centrifuged at 2800 r.p.m. for 10 minutes. A supernatant sample was then carefully removed by pipette and assayed in duplicate.

3.2.9.2 Assay of enzyme extracts

The air segmented flow autoanalyser was arranged as illustrated in the flow diagram (Figure 3.2). Samples of the supernatant (0.25 ml) are sampled and pumped at

a constant rate through the analyser with each sample being separated by a wash with extracting solution. The sample is then mixed with an equal volume of *beta*-limit dextrin solution 33.3 g l^{-1} (Central Laboratory, Rank Hovis and McDougall Ltd, Southampton, UK) and segmented with air. This mixture passes through a mixing coil and is then incubated at 30 °C for 20 minutes. After incubation a small aliquot of the sample mixture is taken and pumped into a stream of iodine solution (0.13 g l^{-1} iodine and 0.39 g l^{-1} potassium iodide in distilled water) passing through a mixing coil. The optical density of the solution is then measured spectrophotometrically in a 10 mm flow cell at 550 nm. Standard (Drift) samples are included after every 6 samples to allow for corrections due to baseline drift of the photometer. The system allows twenty four samples to be analysed in 90 minutes. The photometer records a data trace (Figure 3.3) which is then electronically analysed by computer. High *alpha*-amylase activity in the flour causes a greater breakdown of *beta*-limit dextrin into its constituent sugars leading to a less intense blue/black colouration as fewer polyiodide complexes are formed. Flours with lower *alpha*-amylase activity do not breakdown the *beta*-limit dextrin to such an extent and thus more polyiodide complexes are formed giving an intense blue/black coloured solution. The system was calibrated in Phadebas units using flours of known *alpha*-amylase enzyme activity varying from 20-800 m EU g^{-1} dry weight (one unit (U) of amylase activity is defined as the amount of enzyme catalysing the hydrolysis of 1 µmol glucosidic linkage per minute at 37 °C) as measured using the manual Phadebas assay (Barnes and Blakeney, 1974). The system was recalibrated before each experimental analysis.

In calculating mean *alpha*-amylase activity values, supernatant duplicate values within ±5% of each other, and extraction duplicate values within ±10% of each other were deemed acceptable. Samples were re-analysed if values fell outside this range.

Figure 3.2 : Flow diagram of Skalar air-segmented flow autoanalyser.

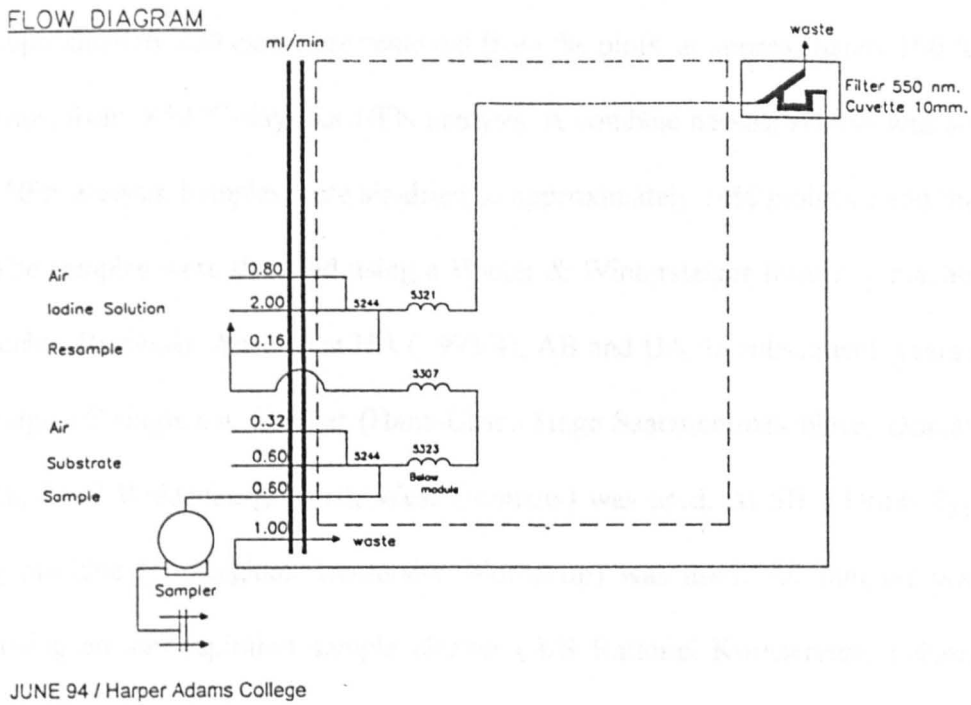
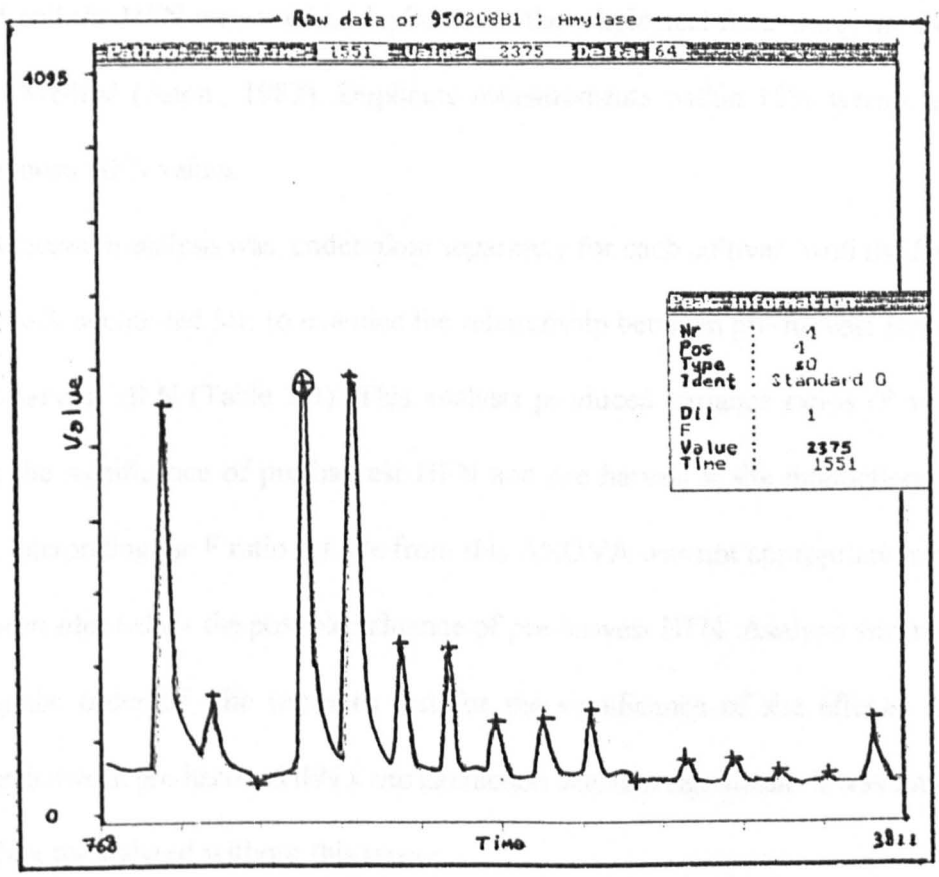


Figure 3.3 : Output from Skalar autoanalyser, with peak height equating to *alpha*-amylase activity.



3.2.10 Hagberg falling number (HFN)

Approximately 250 ears were removed from the plots at approximately 100 °C-day intervals, from 850 °C-days for HFN analysis. A combine harvest sample was also taken for HFN analysis. Samples were air-dried to approximately 14% moisture and then stored. The samples were threshed using a Walter & Wintersteiger threshing machine (Maschinenbau Riedinnkr, Austria) at HA (1993/4), AB and UA. In subsequent years at HA a 'Hege 16' single ear thresher (Hans-Ulrich Hege Saatzuchtmaschinen, Domäne Hohebuch, 7112 Waldenburg/Württ, West Germany) was used. At SB a Drum Type threshing machine (Nottingham University Workshop) was used. All samples were cleaned using an air- aspirated sample cleaner (A/S Rationel Kornservice, Esbjerg, Denmark) fitted with slotted sieves that removed grains above 4.5mm and under 2mm in diameter. The grain was milled using a hammer mill (Falling Number AB, Stockholm, Sweden) and the HFN measured in duplicate on the wholemeal flour using the British Standard Method (Anon., 1982). Duplicate measurements within ±5% were used to calculate mean HFN values.

Regression analysis was undertaken separately for each cultivar, with the factors site and block accounted for, to examine the relationship between pre-harvest HFN and combine-harvest HFN (Table 3.4). This analysis produced variance ratios (F values) allowing the significance of pre-harvest HFN and pre-harvest x site interaction to be assessed. Interpreting the F ratio for site from this ANOVA was not appropriate, since it had not been adjusted for the possible influence of pre-harvest HFN. Analysis was redone reversing the order of the terms to test for the significance of site effects. If the interaction between pre-harvest HFN x site interaction was non-significant it was omitted, and the data re-analysed without this term.

Table 3.4: Accumulated analysis of variance table used to examine the relationship between pre-harvest HFN and combine-harvest HFN for each cultivar.

Change	df
+site/block	2
+site	7
+pre-harvest HFN	1
+pre-harvest HFN x site	7
Error	30
Total	47

3.2.10.1 Relationship between HFN samples harvested by hand and combine-harvest HFN samples

At HA in 1995 hand-HFN samples were taken from all plots on the same day as combine-HFN samples. Regression analysis was then undertaken to examine whether a hand-HFN sample was an accurate representation of a combine-HFN sample. Terms included in regression analysis are shown below (Table 3.5). It was not possible to perform analysis on each cultivar separately due to limited replication in the experiment.

Table 3.5 : Accumulated analysis of variance table used in investigation of relationship between hand-harvested HFN sample and combine-harvest HFN.

Change	df
+block	2
+cultivar	3
+hand-harvested HFN	1
+hand-harvested HFN x cultivar	3
Error	2
Total	11

3.2.10.2 The precision of pre-harvest HFN sampling by hand

The precision of HFN sampling by hand was investigated in 1996 at HA. An additional series of nine HFN samples from the cultivars Hornet and Riband were taken by hand at 850° C-days from three replicate plots and their HFN determined. Initial ANOVA was undertaken on the whole data set of nine samples per replicate. Data from samples was then randomly removed to generate data sets of eight (8a-8e), seven (7a-7e), six (6a-6e), five (5a-5e), four (4a-4e), three (3a-3e), two (2a-2e) and one sample(s) (1a-

1e).This procedure was repeated four times, generating 40 different data sets in total (Table 3.6).

Table 3.6 : An example of the generation of three of the data sets used to investigate the precision of hand-harvest HFN sampling.

Original : 1 2 3 4 5 6 7 8 9

Sample Number 8	Sample Number 7	Sample Number 6
Data Set 8a : 1 2 3 4 5 6 7 8	Data Set 7a : 1 2 3 4 5 6 7	Data Set 6a : 1 2 3 4 5 6
Data Set 8b : 2 3 4 5 6 7 8 9	Data Set 7b : 2 3 4 5 6 7 8	Data Set 6b : 2 3 4 5 6 7
Data Set 8c : 1 2 3 5 6 7 8 9	Data Set 7c : 3 4 5 6 7 8 9	Data Set 6c : 2 3 4 5 6 8
Data Set 8d : 1 2 3 4 5 7 8 9	Data Set 7d : 1 2 4 5 6 7 8	Data Set 6d : 1 2 3 4 6 7
Data Set 8e : 1 3 4 5 6 7 8 9	Data Set 7e : 1 2 3 4 5 6 9	Data Set 6e : 3 4 5 6 7 8

These 40 data sets were then analysed separately using ANOVA and the coefficient of variation for the whole plots (block x cultivar) of each data set was recorded (Table 3.7). A mean coefficient of variation was then calculated for each sample number set (e.g. 8a-8e) using the block x cultivar coefficient of variation.

Table 3.7 : Skeleton analysis of variance table for the precision of sampling HFN by hand.

	df
block	2
block/cultivar stratum	
cultivar	1
Error	2
block/cultivar/sample	48
Total	53

3.2.10.3 Drying and transport of HFN samples harvested by hand

The comparability of drying treatments employed on pre-harvest HFN samples taken at two of the field experimental sites was examined in 1996. Three replicate fresh pre-harvest samples of Hornet and Riband were despatched by overnight courier to HA from UA and AB for centralised drying. ANOVA was then undertaken to identify any relationship between the HFN of centrally dried samples and the HFN of samples dried at field experimental sites (Table 3.8).

Table 3.8 : Skeleton analysis of variance table for the drying and transport of HFN samples harvested by hand.

	df
block	2
block stratum	
site	1
dry	1
cultivar	1
site/block	2
site x dry	1
site x cultivar	1
dry x cultivar	1
site/block x cultivar	4
site/block x dry	4
site x dry x cultivar	1
Error	4
Total	23

3.2.10.4 Pre-harvest HFN sample time point

At HA in 1994 two pre-harvest samples were taken at 827 °C-days and 1025 °C-days (15 and 3 days before the plots were combine-harvested on the 19/08/94) to investigate the effect of sample timing on the relationship between pre-harvest HFN and combine-harvest HFN.

At HA in 1996 HFN samples were taken by hand at 815 °C-days , 937 °C-days and 1066 °C-days (21, 14 and 6 days before combine harvesting on the 02/09/96) to further investigate the effect of sample timing . The data was analysed using regression analysis removing the factors block and cultivar (Table 3.9).

At SB in 1995 a pre-harvest HFN sample was taken early in development after 470 °C-days (44 days before the plots were combined on the 14/08/95). This was to examine whether very early sampling for pre-harvest HFN, still maintained a relationship with combine-harvest HFN.

Table 3.9 : Accumulated analysis of variance table examining the relationship between the HFN of a pre-harvest and combine harvest sample.

Change	df
+block	2
+cultivar	1
+pre-harvest HFN	3
+pre-harvest HFN x cultivar	3
Error	2
Total	11

3.2.11 Fluorescein dibutyrate (FDB) test

This test was used to detect lipase and associated hydrolase activity in grains, to give a qualitative estimate of germination in field material. The test was also used to detect incipient sprouting i.e. sprouting not visible to the naked eye. Grains were mounted crease upper-most on a seed plate and fixed on a cernit clay block (Danbrew Ltd., DK-1801 Frederiksberg C, Denmark) using a seed fixation system (Jensen *et al.*,1984). Two blocks of fifty individual wheat grains were mounted in each operation, with each block consisting of forty-eight test seeds and two germinated wheat seeds exhibiting radicles, which were included as controls. Ninety six seeds from each plot were examined at each sampling time interval. After dorsoventral longitudinal sanding the "half-seeds" obtained were stained for 10 minutes with a solution of fluorescein dibutyrate (ICN Biochemicals, Cleveland, Ohio, USA) (236 mg fluorescein dibutyrate dissolved in 80 ml 99% ethanol made up to 100 ml with distilled H_20). Plates were then soaked in water at 60°C for 2 minutes. Excess water was then dabbed off and the plates viewed under a UV light. The appearance of yellow fluorescent fluorescein in the embryo region of the grain indicated a sprouted wheat grain.

3.2.12 Visual sprouting score

For each sample time-point, 300 grains from 20 ears from each plot were visually

inspected for sprouting. A visually sprouted grain was defined as any grain exhibiting a crack in the pericarp covering the embryo or a radicle (Wellington, 1956).

3.2.13 Iso-electric focusing

A modification of the method developed by Sargeant and Walker (1978) using a Pharmacia Biotech Multiphor electrophoresis unit (Pharmacia Biotech, Uppsula, Sweden) and pH 4.0-6.5 and pH 3.5-9.5 Pharmacia Biotech Ampholine PAGplate polyacrylamide IEF gels was used to distinguish between α-AMY-1 and α-AMY-2 isoenzymes.

3.2.13.1 *Alpha*-amylase extraction

Alpha-amylase was extracted (30 °C for 1 hour) from 0.15 g of milled flour samples (or from an equivalent mass of single grains crushed using a pestle and mortar) using 1 ml of a 20 g l^{-1} sodium chloride / 0.2 g l^{-1} calcium acetate extracting solution (Sargeant and Walker, 1978), with vortex mixing of the sample every 15 minutes. The tube was subsequently centrifuged at 2700 rpm for 10 minutes to remove solid particles. The supernatant was carefully removed by pipetting and heated at 70 °C for 15 minutes to inactivate *beta*-amylase activity. The sample was finally centrifuged at 2700 r.p.m for 10 minutes to remove any remaining solid material and the supernatant used for iso-electric focusing. Samples extracted from clearly germinated grain were used as controls on the gel and run at least every tenth sample.

3.2.13.2 Iso-electric focusing parameters

The anode (+) solution consisted of 0.1 M glutamic acid in 0.5 M phosphoric acid and the cathode (-) solution consisted of 0.1 M *beta*-alanine. Sample aliquots of 20 µl were placed in wells on a sample applicator strip on the Pharmacia Biotech Ampholine PAGplate polyacrylamide IEF gels, which were cooled to 10 °C. The gel was pre-focused and samples allowed to run in. The pH 4.0-6.5 gel was then run at 2000 V / 25 mA for

2.5 hours, the pH 3.0-9.5 gels were run at 1500 V / 50 mA for 1.5 hours.

3.2.13.3 Visualisation of *alpha*-amylase isoenzymes

After iso-electric focusing, the gel was incubated in pre-warmed 1 % Fluka® potato starch solution at 30 °C for 10 minutes. Distilled water was then used to wash the gel twice to remove excess starch. The gel was then rocked gently for 5 minutes in a 0.26 g l^{-1} iodine / 0.78 g l^{-1} potassium iodide solution. The gel underwent a further two washes with distilled water to remove excess iodine solution. *Alpha*-amylase activity was then visualised on the gel as transparent bands against a pale blue-black background, with the intensity of the bands giving an indication of the amount of *alpha*-amylase activity present.

3.2.14 Location of *alpha*-amylase activity using *beta*-limit dextrin in an agarose gel

This assay was developed from an initial method produced by Kalpna Kotecha at Campden and Chorleywood Food Research Association (CCFRA), Chipping Campden, Gloucestershire, UK.

3.2.14.1 Preparation of gels

A stoppered tube was used to mix by shaking, 1 g *beta*-limit dextrin (Rank Hovis Ltd., Central Lab, Solent Flour Mills, Southampton, UK) and 10 ml buffer solution (NaCl = 5 g l^{-1}, $CaCl_2$ = 0.2 g l^{-1}). This solution was then allowed to stand to eliminate air bubbles. A gel solution was then prepared by adding 0.5 g (high gel strength "Electran" molecular biology grade) agarose (BDH, Poole, Dorset, UK) to 50 ml of buffer solution and heating at 60-70 °C on a magnetic hotplate, until the solution cleared. The *beta*-limit dextrin solution was then added to the clear agarose and mixed for 2 minutes at 60°C. A heated glass pipette was then used to transfer 11 mls of the dextrin/agarose solution to pre-heated (60 °C) 9 cm Petri dishes. The solution was spread evenly over the Petri dishes, which were then placed on a levelled surface and allowed to set. Gels were stored

at 4 °C and used within 7 days of preparation.

3.2.14.2 Sample Preparation

Grains were longitudinally sliced through the crease region or transversely sliced a third of the way along the grain from the embryo, using a razor blade or scalpel. The exposed face of fifty grains per plot were then gently placed onto the gel using sharp-pointed tweezers. The gel plates were then incubated at 30 °C for 20 minutes. Sectioned pieces were subsequently carefully removed and the gel flooded with iodine/potassium iodide solution ($I_2 = 6.5\ g\ l^{-1}$, $KI = 19.5\ g\ l^{-1}$) for 1-5 minutes until a pattern was visible. Excess iodine solution was decanted off and the gel viewed using a light-box. Unstained patches on the gel corresponded to areas of *beta*-limit dextrin degradation caused by *alpha*-amylase activity, thus indicating the location of *alpha*-amylase activity in the imprint of the grain. If the percentage of grains in a sample showing crease activity, was greater than the percentage of grains showing embryo activity then PMAA was the likely cause of high *alpha*-amylase activity. Conversely, if the percentage of grains showing embryo activity was higher, PoMS was the likely cause of high *alpha*-amylase activity.

3.2.15 Location of *alpha*-amylase activity using Phadebas substrate in an agarose gel

This assay was developed from an initial method produced by Rachel Seamer at the CCFRA, Chipping Campden, Gloucestershire, UK.

3.2.15.1 Preparation of gels

A 0.65 % agarose / 1 % Phadebas solution was prepared by mixing 0.132 g low melting point agarose (A-5030, Type IX, Sigma) with 10 ml of distilled water on a magnetic stirrer at 50 °C. In a separate beaker 10 ml of water was added to 0.264 g Phadebas powder (Pharmacia Biochem, Uppsala, Sweden). The dissolved agarose was

then added to Phadebas solution and mixed for several minutes on the magnetic stirrer at 50 °C. Aliquots of 2 ml were then pipetted onto clean Petri dishes (60 mm diameter) and left to set on a level surface at 10 °C. Petri dishes were sealed with tape and stored in an airtight container containing damp filter paper to prevent the gels drying out. Gels were used within 7 days of preparation.

3.2.15.2 Sample preparation

Grains were prepared as in Section 3.2.15.2, with 25 grains placed on each plate, and 50 grains per plot analysed. Gel plates were incubated at 40 °C. After incubation the gels were cooled on ice to solidify the gel. Sectioned pieces were then carefully removed and the gel washed carefully with distilled water to remove digested Phadebas dye. Grains demonstrating PoMS were detectable after 15-30 minutes incubation, whereas detection of PMAA took 3-4 hours. The gel was viewed using a light-box. The presence of *alpha*-amylase activity was indicated by clear patches on the gel where the blue Phadebas dye had been digested.

3.2.16 Statistical analysis

Statistical analysis of the data was undertaken using the statistical package GENSTAT, version 5 (Payne *et al.*, 1993).

3.3.3 Results

3.3.1 Weather data

The long term mean temperature (°C) and the long term mean rainfall (mm), between May and September, are illustrated for HA, SB, AB and UA in Table 3.10. The HA data is obtained from the 30 year period between 1961-1990, the AB data from the 30 year period between 1965-1994, whereas for SB the weather records are more

complete allowing a long term average from 1921-1994 to be used to illustrate mean temperature, with data from 1916-1994 used to illustrate mean rainfall. For UA data is obtained from the period 1931-1960. Summary meteorological data for the 1994, 1995 and 1996 are provided in Tables 3.11, 3.12, and 3.13 respectively.

3.3.1.1 Weather in 1994

The summer of 1994 was comparatively warm and dry. The mean July temperature was 1.2 °C above the long term average at SB, whereas at HA the mean July temperature was 1.8 °C above the 30 year mean. The other notable deviation from average temperature occurred at SB where the mean May temperature was 2.7 °C below the long term average. The summer was also comparatively dry with SB receiving only 17% of its average June rainfall, 55% of its average July rainfall and 60 % of its average August rainfall. Similarly HA received only 25% of its average June rainfall and 70% of its average August rainfall.

3.3.1.2 Weather in 1995

The summer can broadly be described as hot and dry. The mean July temperature was 2.1 °C above the long term mean temperatures at HA and AB, 1.9 °C above at SB and 1.5 °C above at UA. Similarly in August mean temperatures were 3.1 °C above the long term mean temperature at HA and SB, 3.5 °C above at AB and 2.2 °C above at UA. Between June and August, SB received 18% of its normal average rainfall, HA received only 21%, AB received 26% and UA received 91%. In September however UA received 6.6 times its normal average rainfall, with 103.2 mm falling on September 11 th alone.

3.3.1.3 Weather in 1996

The summer was warm and dry initially although becoming wetter in August. Two sites, HA and SB, experienced mean temperatures of 0.4-1.0 °C below the long term mean in June and July. The other notable feature is that SB experienced a mean

Table 3.10 : Long term mean temperature (°C) and rainfall (mm) at the four field sites.

Site	Location	Mean temperature (°C)					Total rainfall (mm)				
		Month					Month				
		May	June	July	August	September	May	June	July	August	September
AB	51° 05' N 01° 16' W	11.2	14.8	17.1	16.9	14.3	56.7	57.9	51.3	56.8	65.7
HA	52° 46' N 02° 23' W	10.8	13.8	15.6	15.4	13.1	57.2	54.2	49.1	60.4	56.0
SB	52° 49' N 01° 15' W	12.8	14.9	17.1	15.7	14.1	47	47	51	60	58
UA	57° 18' N 02° 09' W	8.9	11.8	13.7	13.4	11.6	59.0	53.0	60.0	75.0	68.0

temperature 1.3 °C above the long term average in August. AB experienced mean temperatures similar to the long term mean values between June and August. At UA however mean temperatures were 0.7-1.5 °C above average between June and August. The summer was again fairly dry. Between June and July, HA received 50% of its normal average rainfall, SB received 62% and AB received 71%, while UA received 20% more than average. August was a wetter month, with both AB, SB and UA experiencing rainfall amounts similar to their long term means with HA experiencing 35% more rainfall than average. September was a drier month with UA receiving 72% of its normal average rainfall.

3.3.2 Plant development

3.3.2.1. Date of ear emergence

The dates of ear emergence (ZGS 55), illustrated in Table 3.14., demonstrate the wide diversity in cultivar development caused by their response to the environment at different sites over the years of study. The cultivars reached ear emergence earliest in the following year order 1995 > 1994 > 1996, and in the following site order in each of the years of study AB > SB > HA > UA. This reflects the sowing date and the weather conditions at experienced at each geographical location.

At each site the cultivars reached ear emergence in the following order Recital > Soissons > Scipion = Thesee > Pastiche = Riband > Hornet > Haven. The earliest maturing cultivar was Recital in all years, reaching ear emergence 3-8 days before any other cultivar. The other French cultivars all reached ear emergence within 6 days of each other. The UK cultivars reached ear emergence 1-8 days after the latest maturing French cultivar and 5-14 days after Recital. The UK cultivars all reached ear emergence within 7 days of each other. The dates the various cultivars reached the different stages of grain

Table 3.11 : Summary meteorological data from the two field sites in 1994.

Parameter	Month	Site	
		HA	SB
Mean maximum temperature (°C)	May	14.7	*
	June	19.0	19.8
	July	23.4	24.4
	August	19.8	20.9
	September	15.9	16.1
Mean minimum temperature (°C)	May	6.3	*
	June	9.1	9.7
	July	11.3	12.3
	August	10.7	11.6
	September	8.6	9.4
Mean temperature (°C)	May	10.5	*
	June	14.0	14.8
	July	17.4	18.3
	August	15.2	16.3
	September	12.3	12.8
Total rainfall (mm)	May	37.6	*
	June	13.8	8.2
	July	42.6	28.4
	August	42.6	33.2
	September	93	106.6
Mean radiation ($Wh\ m^{-2}$)	May	3827	*
	June	4470	5027
	July	5111	4944
	August	3519	3724
	September	2291	2361

* - not recorded

Table 3.12 : Summary meteorological data from the four field sites in 1995.

Parameter	Month	Site			
		HA	SB	AB	UA
Mean maximum temperature (°C)	May	16.7	*	18.6	13.6
	June	19.4	19.1	21.2	15.7
	July	23.6	25.1	26.4	19.7
	August	24.9	25.3	28.5	21.0
	September	*	*	*	15.2
Mean minimum temperature (°C)	May	5.9	*	5.5	5.1
	June	8.3	9.5	8.1	7.9
	July	11.9	12.8	12.1	10.9
	August	12.0	12.4	12.2	10.1
	September	*	*	*	9.2
Mean temperature (°C)	May	11.3	*	12.1	9.4
	June	13.9	14.3	14.7	11.8
	July	17.7	19.0	19.2	15.2
	August	18.5	18.8	20.4	15.6
	September	*	*	*	11.9
Total rainfall (mm)	May	37.2	*	28.4	97
	June	14.8	11.7	15.0	88.7
	July	13.2	10.7	22.2	60.1
	August	7.0	5.7	5.8	22.4
	September	*	*	*	450.8
Mean radiation ($Wh\ m^{-2}$)	May	4348	*	5275	*
	June	5022	4611	5320	*
	July	4796	4583	5130	*
	August	6998	4472	5195	*
	September	*	*	*	*

* - not recorded

Table 3.13 : Summary meteorological data from the four field sites in 1996.

Parameter	Month	Site			
		HA	SB	AB	UA
Mean maximum temperature (°C)	May	*	*	15.1	*
	June	19.0	20.2	21.8	17.0
	July	20.7	22.3	24.1	18.7
	August	21.3	22.1	23.3	18.8
	September	17.6	*	*	16.1
Mean minimum temperature (°C)	May	*	*	4.3	*
	June	7.6	8.1	7.6	8.9
	July	8.5	11.0	10.1	10.0
	August	10.1	11.8	10.5	11.0
	September	7.5	*	*	9.1
Mean temperature (°C)	May	*	*	9.7	*
	June	13.3	14.1	14.7	12.9
	July	14.6	16.7	17.1	14.4
	August	15.6	17.0	16.9	14.9
	September	12.6	*	*	12.6
Total rainfall (mm)	May	*	*	48.8	*
	June	33.7	31.2	12.6	31.6
	July	17.9	30.1	65.4	103.8
	August	82.0	58.1	58.0	76.7
	September	16.0	*	*	49.3
Mean radiation (Wh m^{-2})	May	*	*	4565	*
	June	3886	5530	4475	3472
	July	4597	5287	6113	2679
	August	3459	3942	5078	2885
	September	2617	*	*	2778

*- not recorded

development reflected their initial ear emergence and anthesis date.

Anthesis of the cultivars (ZGS 65) occurred 119-164 °C-days (8-12 days) after ZGS 55 in 1994, 118-173 °C-days (10-14 days) in 1995 and 71-123 °C-days (4-9 days) in 1996. The dates various ZGS were reached by the four UK cultivars at HA between 1994 -1996 are shown in Appendix 3. There was little difference in the rates the cultivars reached maturity at the particular sites, although Hornet was the slowest maturing UK cultivar. All the cultivars reached caryopsis hard stage (ZGS 92) at approximately 1100 °C-days after ear emergence. Weather conditions and the availability of the combine harvester dictated when the plots were actually combined after this point.

3.3.3 Moisture content and grain drying-rate

Figures 3.4-3.7 illustrate the change in moisture content of the grains from florets 1 and 2 of the central spikelets of the four UK cultivars at the ten site x year combinations. All the crops reached a commercial combinable moisture content of 14% at approximately 1100 °C-days after heading.

3.3.3.1 Moisture content of the cultivar Haven

In 1994, Haven behaved similarly to Hornet, having a higher moisture content between 600-1000 °C-days at HA compared to SB (Figure 3.4). In 1995 moisture loss was similar at HA, AB and SB. At UA moisture content remained higher later on in the developmental timescale, with adverse weather preventing harvesting of the grain. In 1996 moisture content curves were similar up to 700 °C-days at all sites, but with UA again showing the tendency to have a higher moisture content later in the developmental timescale.

Table 3.14 : Differences in the calendar dates of heading (ZGS 55) at the four field experiment sites over the period 1994-1996.

Cultivar	HA			SB			AB		UA	
	1994	1995	1996	1994	1995	1996	1995	1996	1995	1996
Recital	31 May	24 May	8 Jun	26 May	21 May	3 Jun	15 May	*	18 Jun	*
Soissons	4 Jun	29 May	11 Jun	31 May	27 May	8 Jun	23 May	4 Jun	21 Jun	21 Jun
Scipion	6 Jun	29 May	12 Jun	5 Jun	26 May	9 Jun	24 May	5 Jun	23 Jun	24 Jun
Thesee	6 Jun	30 May	13 Jun	3 Jun	27 May	8 Jun	24 May	5 Jun	22 Jun	25 Jun
Pastiche	14 Jun	7 Jun	16 Jun	8 Jun	31 May	12 Jun	31 May	8 Jun	24 Jun	26 Jun
Riband	15 Jun	7 Jun	16 Jun	8 Jun	30 May	12 Jun	31 May	8 Jun	25 Jun	29 Jun
Hornet	15 Jun	9 Jun	17 Jun	11 Jun	3 Jun	13 Jun	3 Jun	11 Jun	27 Jun	29 Jun
Haven	16 Jun	9 Jun	17 Jun	12 Jun	5 Jun	13 Jun	6 Jun	12 Jun	27 Jun	1 Jul

* - Recital plots not monitored due to poor viability seed being sown.

3.3.3.2 Moisture content of the cultivar Hornet

In 1994, Hornet had a higher moisture content between 600-1000 °C-days at HA compared to SB. In 1995 moisture loss was similar at AB and UA, but it was more rapid at HA and SB. In 1996 moisture content curves were similar up to 700 °C-days, with higher moisture contents later in the development than previous years seen (Figure 3.5).

3.3.3.3 Moisture content of the cultivar Pastiche

In 1994, at both HA and SB, there was a steady fall in the moisture content of the grains during development with no significant difference in the moisture loss at the two sites (Figure 3.6). In 1995 the moisture loss was similar at SB, AB and UA up to 700 °C-days, with the HA crop showing a lower moisture content than the other sites throughout grain development. The curves then diverged with severe weather preventing the drying and harvesting of the grain at UA. At SB, AB and HA similar moisture content curves were seen. In 1996 the moisture content curves were again similar up to 800 °C-days at all sites, with only UA diverging and retaining a moisture content of around 40% between 700-1100 °C-days.

3.3.3.4 Moisture content of the cultivar Riband

In 1994, the moisture content curves were very similar although moisture content was lower at SB between 700-900 °C-days as compared to HA (Figure 3.7). In 1995 HA and SB showed similar rapid moisture loss throughout development. At AB and UA moisture contents were higher during development. The severe wet weather at UA (404.8 mm of rain fell between September 1 st and 12 th) caused PoMS, leading to an HFN of 62. At AB moisture loss was slower than that at HA and SB. In 1996 moisture content curves were again similar up to 700 °C-days. There was then a wide disparity in the curves. HA declined most rapidly, despite a rise in moisture content before harvest due

Figure 3.4 : The change in percentage moisture content of the cultivar Haven during grain development at ten site x year combinations between 1994-1996.

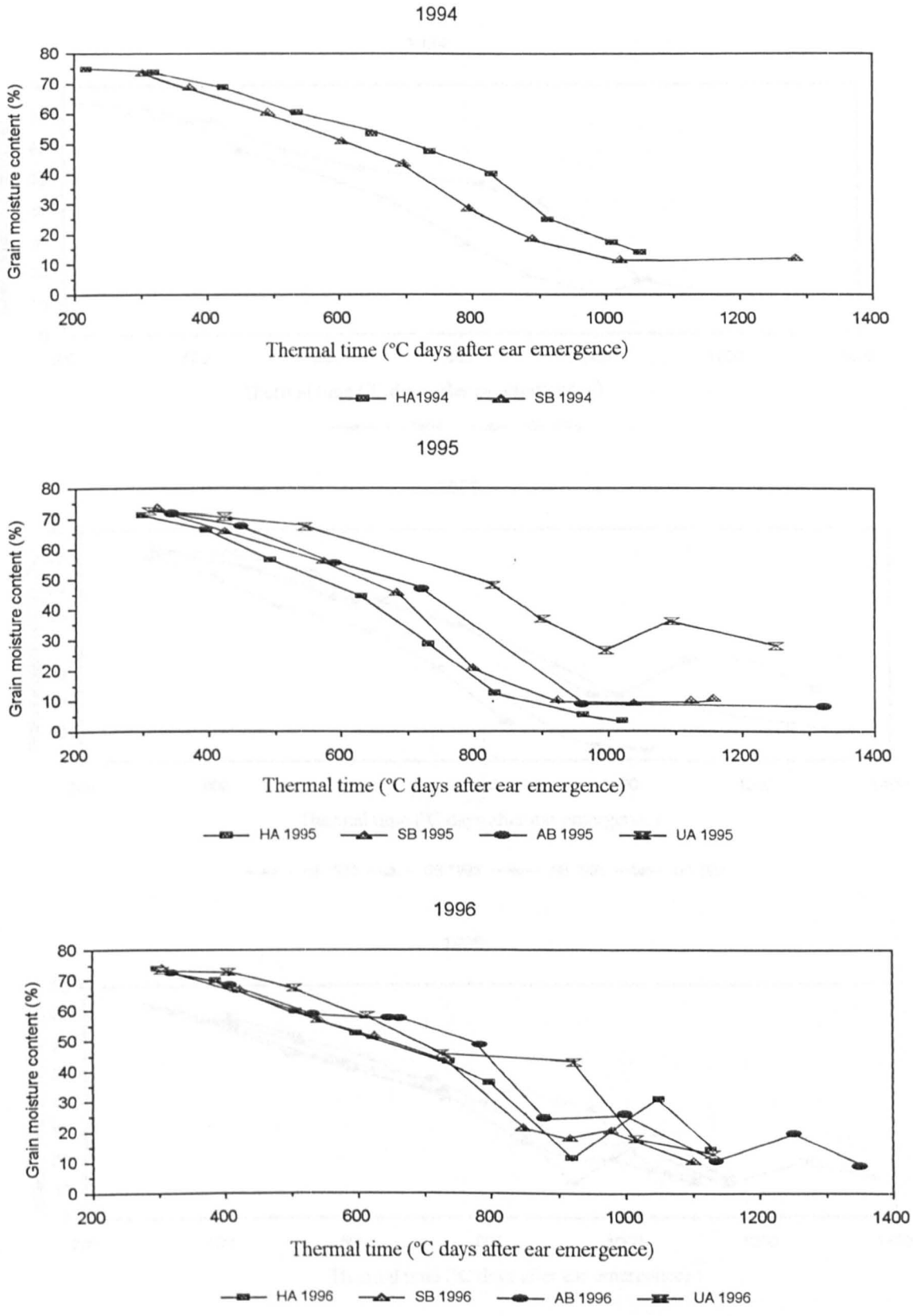


Figure 3.5 : The change in percentage moisture content of the cultivar Hornet during grain development at ten site x year combinations between 1994-1996.

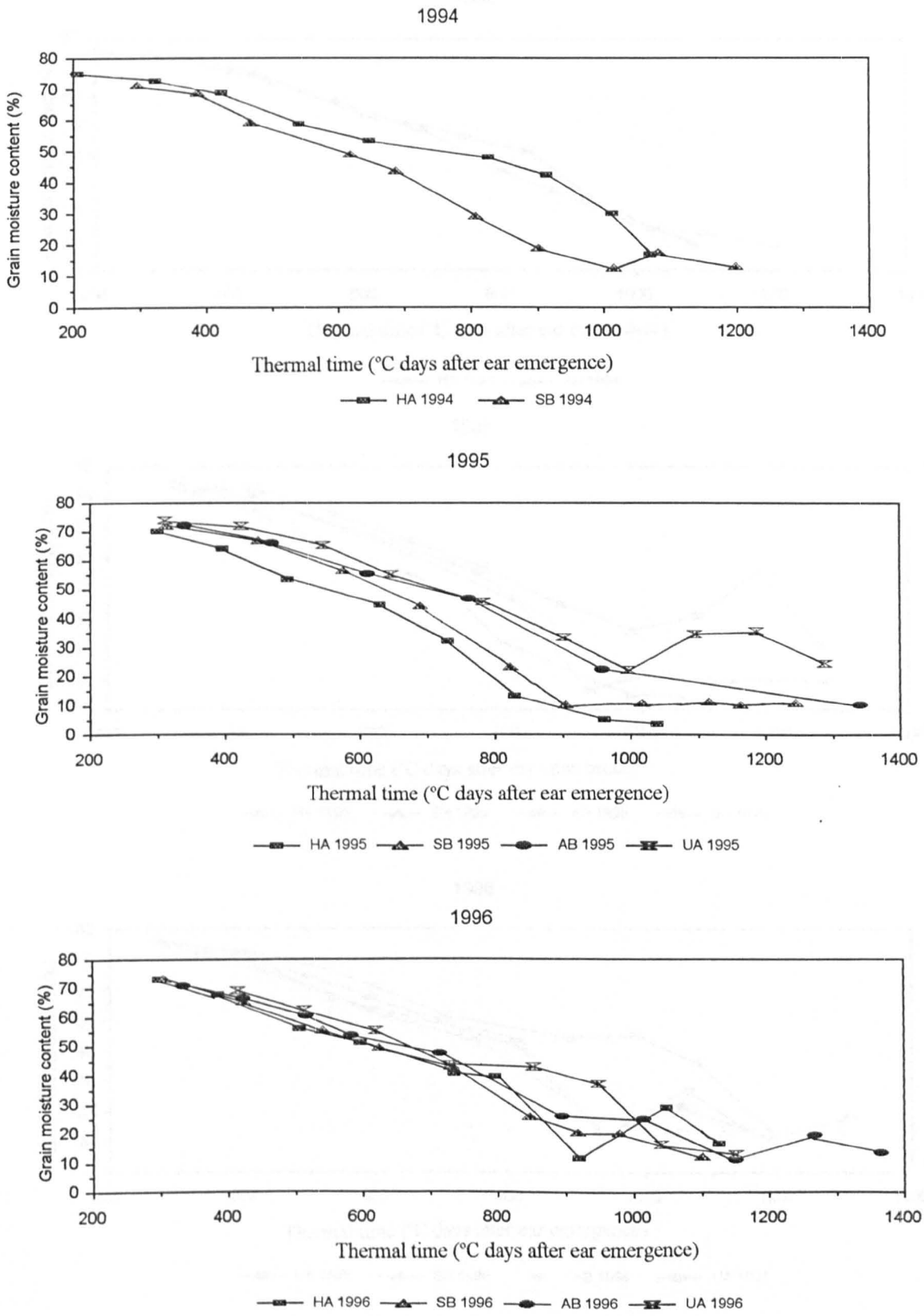


Figure 3.6 : The change in percentage moisture content of the cultivar Pastiche during grain development at ten site x year combinations between 1994-1996.

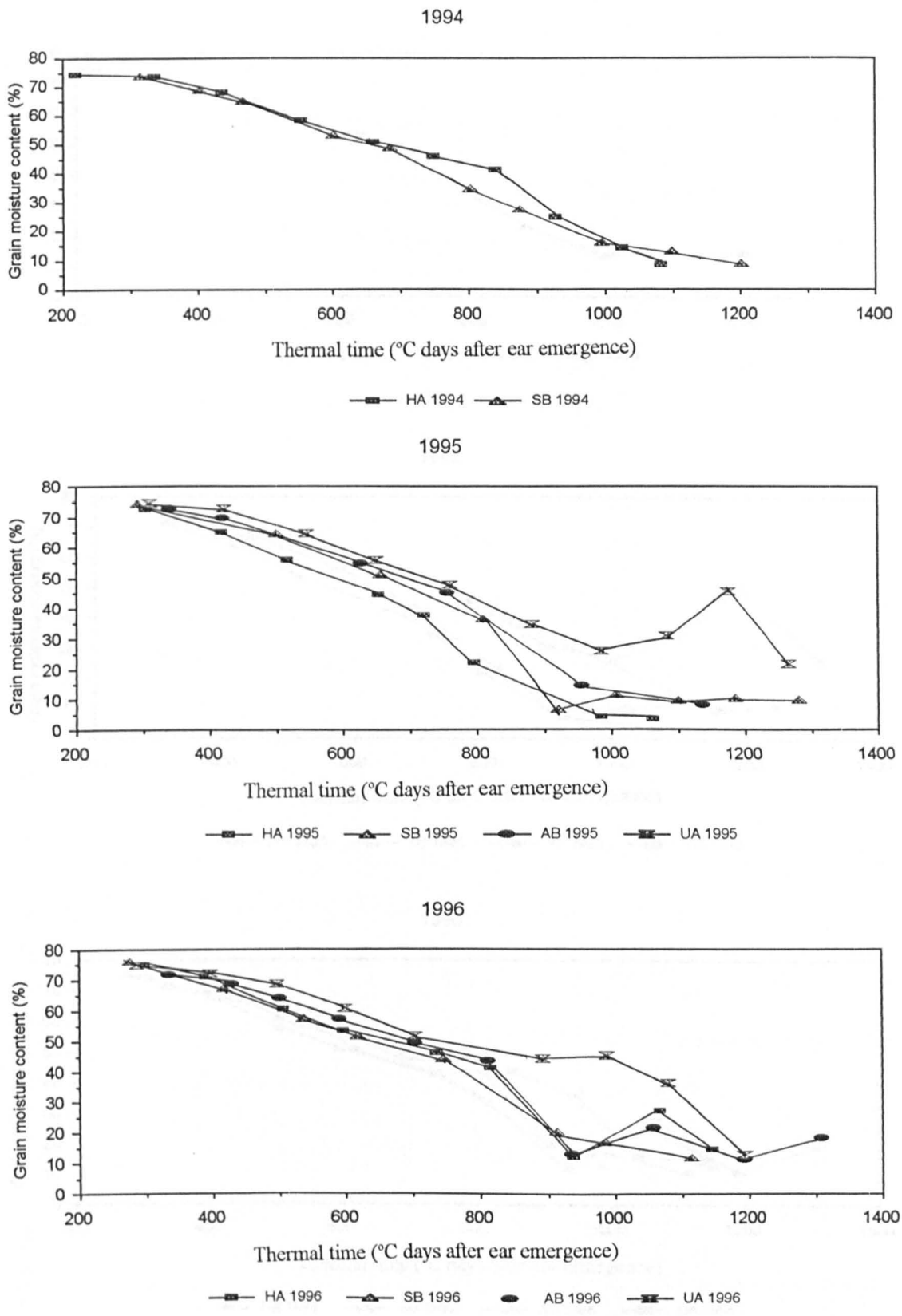
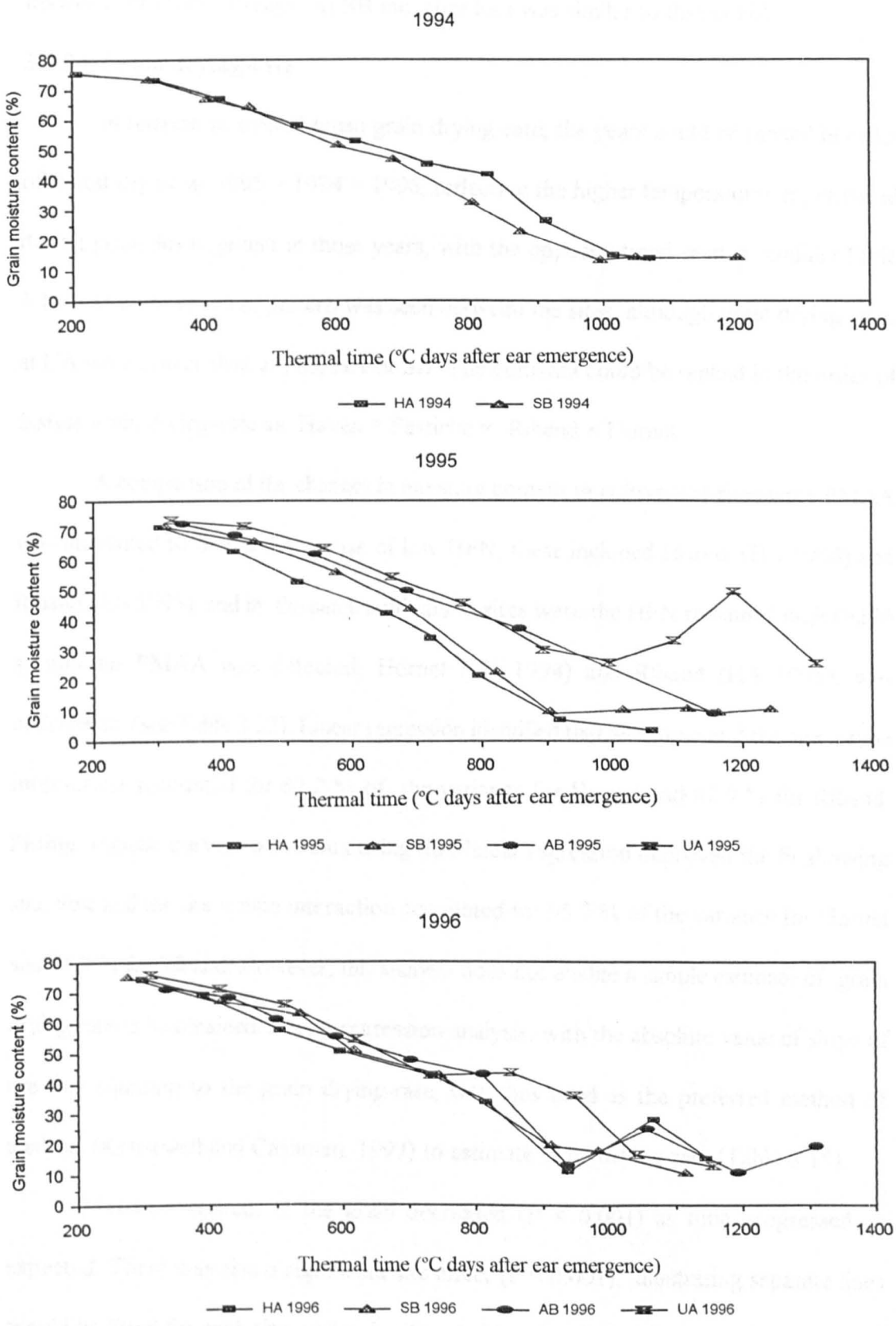


Figure 3.7: The change in percentage moisture content of the cultivar Riband during grain development at ten site x year combinations between 1994-1996.



to rain. A similar situation occurred at AB. At UA moisture content again remained higher between 700-1000 °C-days. At SB moisture loss was similar to that at HA.

3.3.3.5 Grain drying-rate

In relation to overall mean grain drying-rate, the years could be ranked in order of fastest drying as 1995 > 1994 > 1996, reflecting the higher temperatures experienced during grain development in those years, with the opposite trend seen in rainfall (Table 3.11-3.13). A less clear pattern was seen between the sites, although grain drying-rates at UA were slower than at AB, HA or SB. The cultivars could be ranked in the order of fastest grain drying-rate as Haven > Pastiche = Riband = Hornet.

A comparison of the changes in moisture content in cultivars at sites were PMAA was attributed to be the sole cause of low HFN, these included Hornet (HA 1994) and Riband (AB 1995), and in the same cultivars at sites were the HFN remained high (>250 s) and no PMAA was detected, Hornet (SB 1994) and Riband (HA 1995), was undertaken (see Table 3.22). Linear regression identified that site, time and the site x time interaction accounted for 82.2 % of the variance for Hornet and 86.9 % for Riband. Fitting logistic curves to the data using non-linear regression improved the fit showing site, time and the site x time interaction accounted for 95.7 % of the variance for Hornet and 94.9 % for Riband. However, this analysis does not enable a simple estimate of grain drying-rate to be obtained. Linear regression analysis, with the absolute value of slope of the line equating to the grain drying-rate, was thus used as the preferred method of analysis (Kettlewell and Cashman, 1997) to estimate grain drying-rate (Table 3.15).

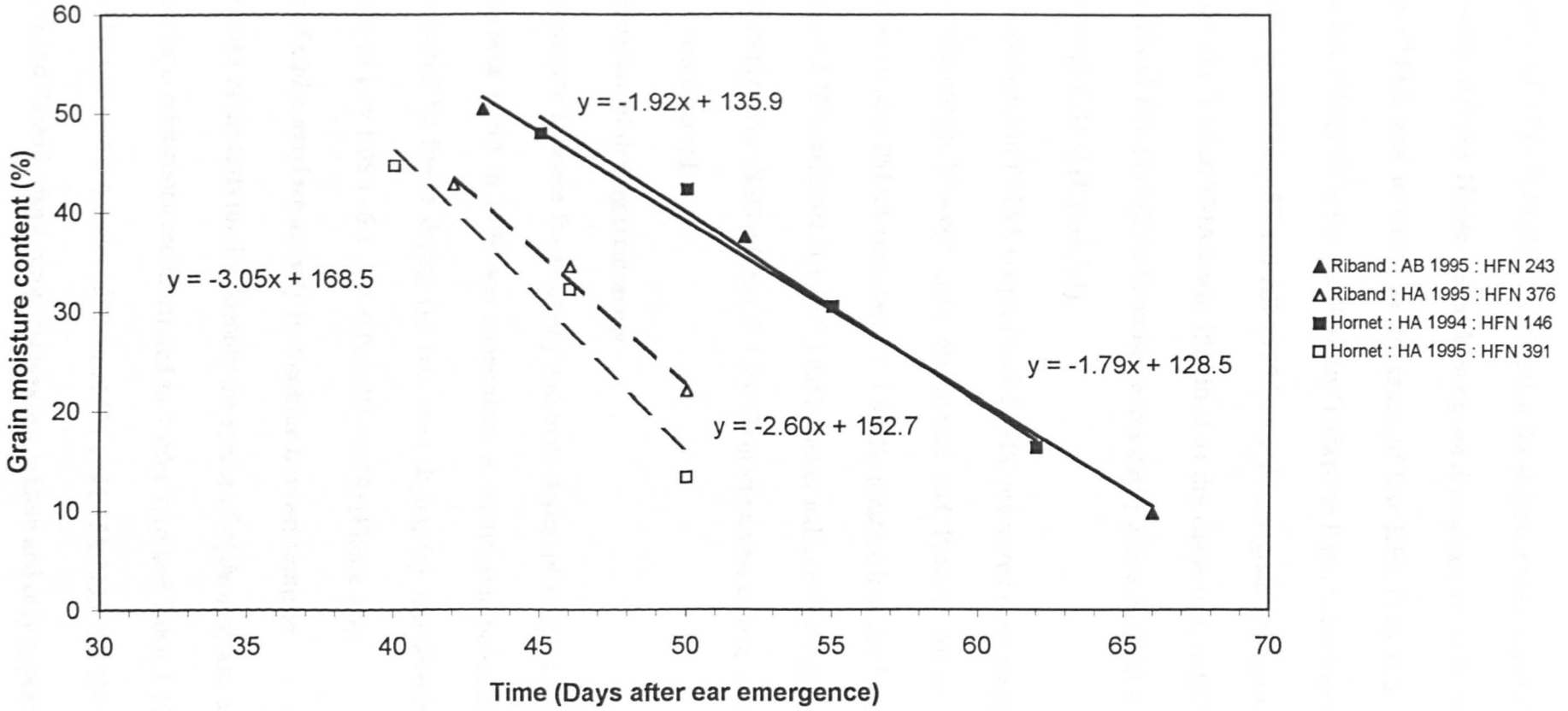
Moisture content in the grain decreased ($P < 0.001$) as time progressed as expected. There was also a significant site effect ($P < 0.001$), illustrating separate lines should be fitted for each site, and a significant site x time interaction ($P = 0.002$ for

Table 3.15 : Mean grain drying-rate (% moisture loss day $^{-1}$) between 50-20 % moisture content of UK cultivars from field experimental sites in 1994, 1995 and 1996.

Cultivar	1994		1995				1996			
	HA	SB	HA	SB	AB	[1]UA	HA	SB	AB	UA
Haven	1.98	2.56	3.41	2.16	3.18	1.58	1.93	3.24	2.49	1.24
Hornet	1.92	2.47	3.05	1.82	2.44	1.69	1.93	2.11	2.42	1.18
Riband	1.83	2.11	2.60	2.72	1.79	1.57	2.00	2.28	2.70	1.18
Pastiche	1.85	2.16	2.98	2.01	2.16	1.41	1.96	2.44	2.83	1.14
Overall Mean	1.88	2.37	3.24	2.18	2.30	1.56	1.95	2.52	2.61	1.21
SED (6 df)	0.020	0.147	0.457	0.230	0.084	0.050	0.077	0.218	0.134	0.079
CV (%)	1.3	7.8	17.3	13.0	4.4	4.0	4.8	10.6	6.3	7.9

[1] = Samples not combine-harvested due to severe sprouting.

Figure 3.8 : A comparison between grain drying-rates at sites where PMAA was identified as the cause of the HFN falling below 250 s and at sites where no PMAA was detected.



Hornet, and P = 0.037 for Riband) indicating that the slopes of the moisture loss curves were different for each site. Hornet had a slower grain drying-rate (1.92 % moisture loss day^{-1}) when PMAA was attributed as the cause of low HFN (146 s) at HA 1994, compared to HA 1995 (3.05 % moisture loss day^{-1}) where no PMAA was found and HFN was high (391 s). Similarly Riband-AB-1995 had a slower grain drying-rate (1.79 % moisture loss day^{-1}) when PMAA was identified as the cause of low HFN (243 s), compared to Riband-HA-1995 (2.60 % moisture loss day^{-1}) where no PMAA was found and HFN was high (376 s) (Figure 3.8).

The occurrence of PMAA coupled with PoMS, prevented the investigation of a quantitative relationship between grain drying-rate and PMAA. An overall t-test comparing cases where PMAA was (mean = 1.90 % moisture loss day^{-1}) or was not detected (mean = 2.30 % moisture loss day^{-1}) did however indicate that the grain drying-rate was significantly lower (SED = 0.204, P = 0.047) in site x year cultivar combinations where PMAA was detected.

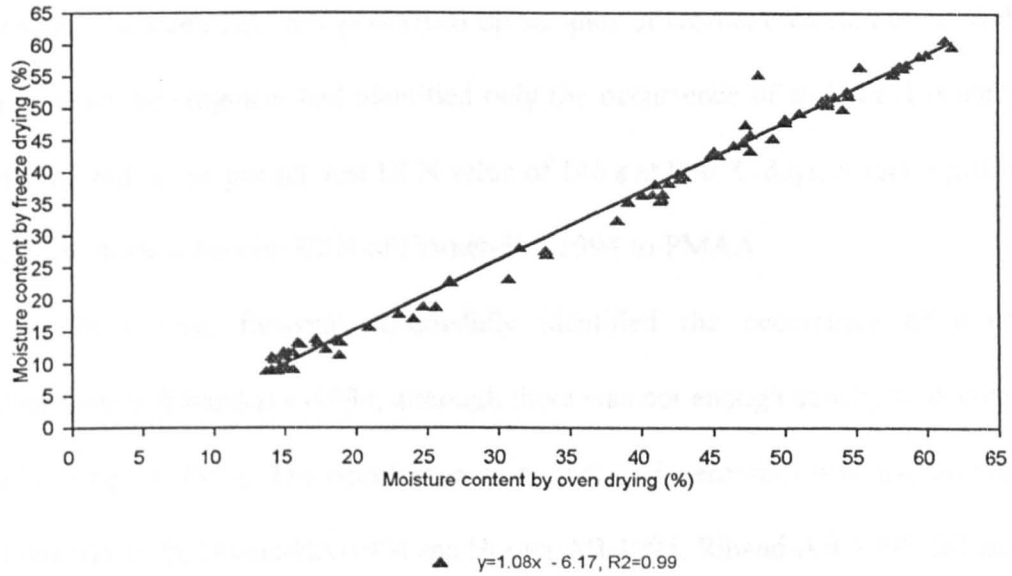
3.3.3.6 Comparison of drying treatments

A comparison between freeze-drying and oven-drying of samples taken during grain development at HA in 1994 was undertaken. A relationship between moisture content determined by freeze drying (y) and oven drying (x) was identified using regression analysis ($y = 1.08\,x - 6.1$, $P < 0.001$, $R^2 = 0.99$) (Figure 3.9).

3.3.4 Source of *alpha*-amylase activity in combine harvest samples

The results of the tests used to identify the source of *alpha*-amylase activity at the 10 site x year combinations are illustrated in Tables 3.16 and Table 3.17. Visual sprouting (PoMS) was seen in samples of Haven at HA 1994, UA 1995, SB 1996 and UA 1996. In Hornet and Riband, visual sprouting was less evident and only appeared at UA

Figure 3.9 : A relationship between the moisture content of grains determined by freeze drying (y) and oven drying (x) during grain development.



1995, SB 1996 and UA 1996, with sprouting in Pastiche only visible at UA 1995.Visual sprouting was generally low in all samples except at UA 1995, where 404.8 mm of rain fell between 1 st and 12 th September, causing even the highly resistant cultivar Pastiche to sprout in more than 50% of the grains. The occurrence of visual sprouting in the cultivars at the ten site x year combinations can be ranked in the following order :- Haven > Hornet ≥ Riband > Pastiche, although some anomalies were seen, *e.g.* SB 1996 where Riband showed higher sprouting than Haven (4.0% *cf.* 3.0%).

Fluorescein dibutyrate staining identified PoMS in four samples (Haven AB 1995 and in Haven, Hornet and Riband at HA 1996) where visual inspection failed to identify it, with two of these cases being where the HFN fell below 250 s (Hornet-HA-1994 and Haven-AB-1995). This validates the usefulness of this technique in determining the source of *alpha*-amylase activity in the grain. Contamination of the harvest sample of

Hornet-HA-1994 with a few grains of Recital caused an initial positive FDB result. On analysing a sample where the grains of Recital had been removed no FDB staining was seen. Furthermore IEF gels performed on samples of Hornet collected by hand during early grain development had identified only the occurrence of α-AMY-1 isoenzymes. Coupled with a low pre-harvest HFN value of 146 s at 850 °C days, it seems justified to attribute the low harvest HFN of Hornet-HA-1994 to PMAA.

Iso-electric focusing successfully identified the occurrence of α-AMY-2 isoenzymes in Riband-HA-1994, although there was not enough activity to decrease the HFN to below 250 s. The occurrence of α-AMY 1 isoenzymes was also identified in Haven-AB-1995, Hornet-HA-1994 and Hornet-AB-1995, Riband-AB-1995, Riband-AB-1996 and Riband-UA-1996, although the bands often appeared rather faint on the gel reflecting the low *alpha*-amylase activity in the samples. The bands of some samples in which visual sprouting had already been identified (Haven-HA-1994, Haven-UA-1996, Hornet-UA-1996) showed up as broad smears on the gel and with both groups of isoenzymes identifiable. Both the *beta*-limit dextrin gel assay and Phadebas gel assay gave good qualitative indications of the location of *alpha*-amylase activity in the grain and were more sensitive than the FDB test (Table 3.17). An outline or "halo effect" (shown up as a clear patch on the gel) around transverse grain sections identified the occurrence of RPAA. On the other hand a clear spot on the gel located around the crease region of the grain identified PMAA. Large clear patches on the gel around the aleurone layer area and crease region of the grain identified the source of *alpha*-amylase as PoMS.

Table 3.17 illustrates the results from the *beta*-limit dextrin gel tests undertaken mainly on samples in which visual sprouting was absent and where no FDB staining had been seen (Table 3.16). Samples from HA 1995 and SB 1995 did not show any detectable

Table 3.16 : Results of tests identifying the source of *alpha*-amylase activity in combine harvest samples at ten site x year combinations from 1994-1996.

Cultivar	Test	1994		1995				1996			
		HA	SB	HA	SB	AB	UA	HA	SB	AB	UA
Haven	Sprouting (%)	1	0	0	0	0	>50	0	2.6	0	0.5
	FDB (%)	2.1	-	-	-	2.7	-	2	-	0	-
	IEF	α-AMY-1 α-AMY-2	-		-	α-AMY-1 (faint)	-	-	-	α-AMY-1 α-AMY-2	α-AMY-1 α-AMY 2
Hornet	Sprouting (%)	0	0	0	0	0	>50	0	3.0	0	0.33
	FDB (%)	1.4 [1]	-	0	-	0	-	2.67	-	0	-
	IEF	α-AMY-1	-	-	-	α-AMY-1 (faint)	-	-	-	α-AMY- 1 α-AMY-2	α-AMY-1 α-AMY-2
Riband	Sprouting (%)	0	0	0	0	0	>50	0	4.0	0	0.33
	FDB (%)	0	-	0	-	0	-	0.33	-	0	-
	IEF	α-AMY 2 (faint)	-	-	-	α-AMY 1 (faint)	-	-	-	α-AMY 1 (faint)	α-AMY 1 (faint)
Pastiche	Sprouting (%)	0	0	0	0	0	>50	0	0	0	0
	FDB (%)	0	-	0	-	-	-	0	-	0	0
	IEF	-	-	-	-	-	-	-	-	-	-

- Sample not analysed.

[1] Positive FDB result caused by contamination of combine harvest sample with some sprouted grains of Recital.

Table 3.17: Results of *beta*-limit dextrin gel tests for identifying the source of *alpha*-amylase activity in combine harvest samples at ten site x year combinations from 1994-1996.

Cultivar	Location of *alpha*-amylase activity	Percentage of grains showing *alpha*-amylase activity in specific regions of grain (%)									
		1994		1995				1996			
		HA	SB	HA	SB	AB	UA	HA	SB	AB	UA
Haven	Embryo	[1]-	[3]-	0	0	[1]-	[1]-	20	[1]--	18	34
	Crease		-	0	0		-	5	-	24	18
Hornet	Embryo	[2] Crease region	[3]-	0	0	2	[1]-	9	[1]--	16	10
	Crease		-	0	0	24	-	7		41	70
Riband	Embryo	0	[3]-	0	0	0	[1]-	7	[1]-	5	14
	Crease	6		0	0	10		5		35	44
Pastiche	Embryo	0	[3]-	0	0	0	[1]-	0	0	0	[3]-
	Crease	2		0	0	0		0	0	0	

[1] -Sample not analysed due to the presence of visual sprouting or positive FDB result.

[2]- Sample analysed by CCFRA, no quantitative data available.

[3] - Sample unavailable for analysis

alpha-amylase activity following *beta*-limit dextrin gel tests. Similarly, the cultivar Pastiche did not show any detectable *alpha*-amylase activity at any of the sites, except for a couple of isolated grains at HA 1994.

In Haven-HA-1996, a high percentage (20%) of grains showed activity in the embryo region of the grain, with a lesser number (5%) of grains showing activity in the crease region. In contrast, in Haven-AB-1996 a grains showed *alpha*-amylase activity in both the embryo (18%) and crease (24%) regions at similar levels, whereas in Haven-UA-1996 a higher number of grains (34%) showed activity in the embryo region compared to the crease region (18%).

Samples from Hornet-HA-1994, were initially analysed by CCFRA and only a qualitative result was available, but samples clearly showed activity in the crease region of the grain. In Hornet-AB-1995, a high percentage of grains showed *alpha*-amylase activity in the crease region (24%) with only 2% of grains showing activity in the embryo region. In Hornet-UA-1996, the percentage of grains showing activity in the embryo (9%) and crease (7%) regions was similar. In contrast, in Hornet-AB-1996 a very high percentage of grains showed crease activity (41%), but a high proportion of grains also showed activity in the embryo region (16%). In samples of Hornet-UA-1996, a massive 70% of grains showed activity in the crease region, with 10% of grains showing activity in the embryo region.

In Riband-HA-1994 and Riband-AB-1995, activity was only seen in the crease region of the grains, although this activity was at the low levels of 6% and 10% of grains respectively. A low proportion of grains also showed activity in the crease region (5%) in Riband-HA-1996, although activity was also seen in the embryo region (7%) in a pattern similar to that seen in Hornet-HA-1996. In Riband-AB-1996, a high proportion

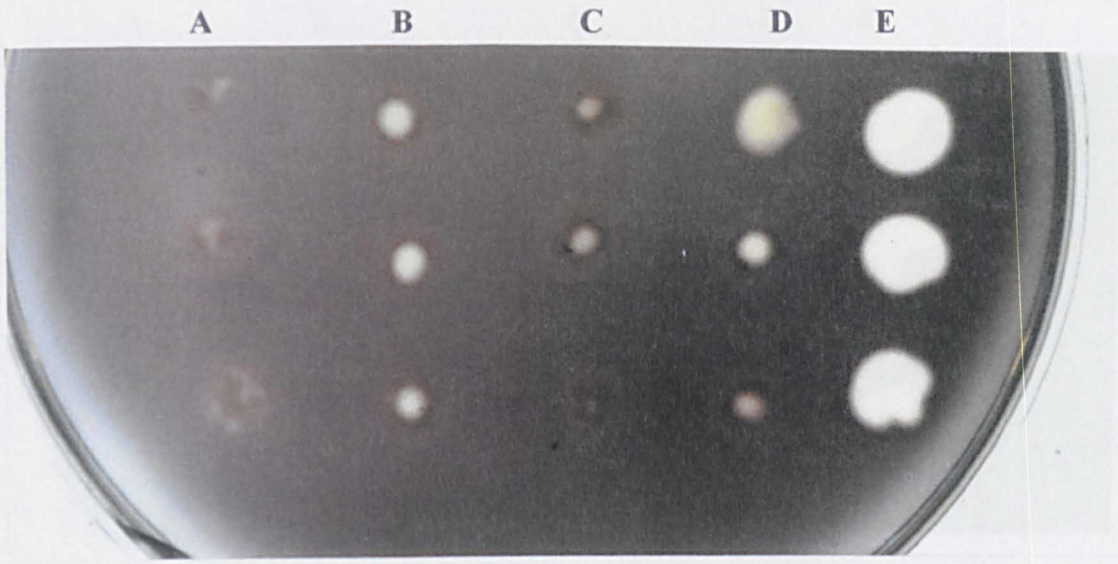
of grains showed activity in the crease region (35%) compared to a low level of grains showing activity in the embryo region (5%) A similar pattern was seen in Riband-UA-1996, with a high proportion of grains showing activity in the crease region (44%) with a lower percentage showing activity in the embryo region (14%), which was similar to the pattern of activity seen in Hornet-AB-1996. Plates 3.2 and Plate 3.3 illustrate some examples of the results from the *beta*-limit dextrin gels.

Plate 3.2 illustrates the location of *alpha*-amylase activity in transverse sections of grain from Hornet-UA-1996 (PMAA +PoMS) and Hornet- HA-1994 (PMAA). Lane A contains green immature grains of Riband, with a characteristic halo-effect appearing around the grain imprint on the gel due to *alpha*-amylase activity in the pericarp. Lane B contains combine-harvested grains from Hornet UA 1996 showing *alpha*-amylase activity located only in the crease region in these particularly sampled grains, a characteristic of PMAA. Other grains in the Hornet UA 1996 combine sample also showed gel patterns similarly to Lane E, which contains visibly sprouted grains of Pastiche, which act as PoMS controls on the gel. These sprouted grains formed a large clear patch on the gel as transverse sectioning exposes the endosperm "face" of the grain to the gel, which is undergoing degradation by *alpha*-amylase activity as PoMS progresses. Lane C contains combine-harvested grains of Hornet-HA-1994, illustrating *alpha*-amylase activity located solely around the crease region of the grain characteristic of PMAA. Lane D contains grains of Pastiche germinated in the laboratory for 48 hours to illustrate the pattern obtained in sprouting grains. The gel pattern obtained in Lane D is very similar to Lanes B and C, with activity located around crease region of the grain. This illustrates that differentiating between PMAA and PoMS using solely transverse sectioning is not possible as the early stages of PoMS appear similar to PMAA. Longitudinal sectioning of

the grains allows a better distinction.

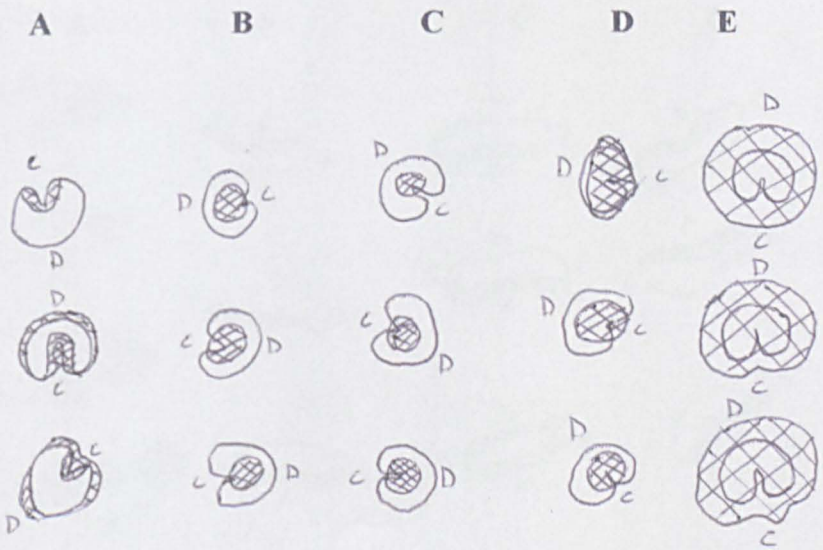
Plate 3.3 illustrates the location of *alpha*-amylase activity in longitudinal sections of grain from UA 1996 Hornet (PMAA + PoMS) and HA 1994 Hornet (PMAA). Lane A contains grains of Pastiche germinated in the laboratory for 48 hours, with a large clear patch in the gel located at the embryo end of the grain indicating *alpha*-amylase activity due to PoMS. In contrast, in Lane B these particular grains from Hornet UA 1996 only showed clear patches along the exposed crease region of the gel, characteristic of PMAA. Other grains from this sample also showed patterns similar to grains in control Lane E. Lane E contained visibly sprouted grains, with a large clear patch obtained on the gel indicating *alpha*-amylase activity was located throughout the grain endosperm indicating PoMS was occurring. Lane D contained combine-harvest grains of Hornet HA 1994, with faint clear patches occurring on the gel around the crease region. This again suggests PMAA is causing the high alpha-amylase activity in Hornet HA 1994, as no clear patches were seen at the embryo end of the grain indicating PoMS is not occurring. Lane C contains green immature grains of Riband, with a halo like effect agin occurring around the grains due to *alpha*-amylase activity located in the pericarp, this acts as a control on the gel for RPAA. The ratio of grains showing embryo or crease activity gave a good indication of the source of *alpha*-amylase in particular samples. If the percentage of grains showing crease activity was greater than or equal to the number of grains showing embryo activity, then PMAA was the most probable cause of high *alpha*-amylase activity in a sample. Alternatively, if the percentage of grains showing crease activity was less than the number of grains showing embryo activity then PoMS was the most probable cause. Trying to quantify these visual indications of *alpha*-amylase activity with actual HFN did not reveal any firm relationships. Analysis of a greater number of grains may

Plate 3.2 : *Beta*-limit dextrin gel illustrating the location of *alpha*-amylase enzyme activity in transverse sections of grains of Hornet from UA in 1996.



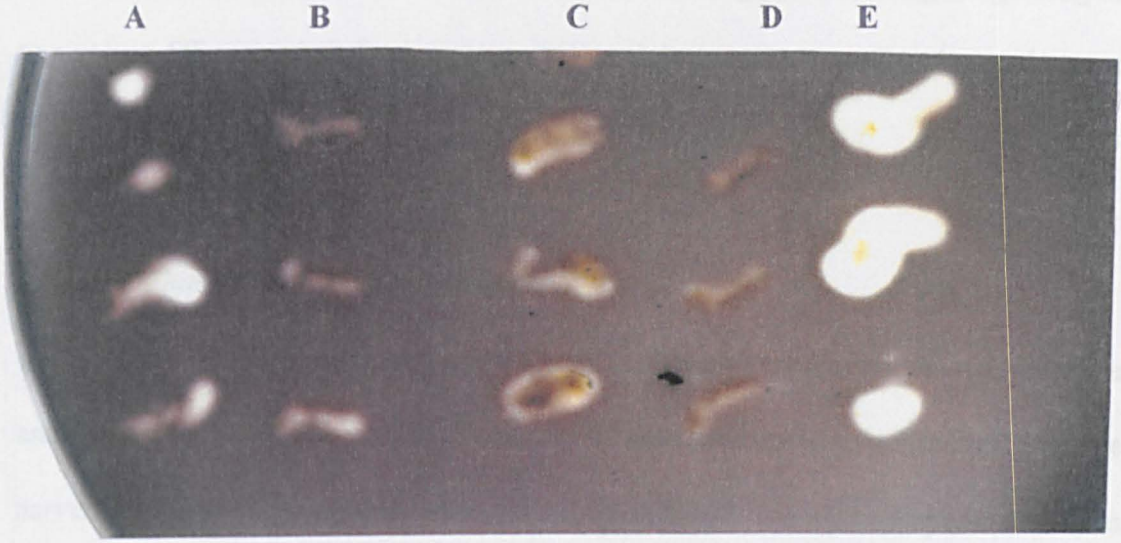
Key : A : Control : Riband-HA-1996 immature grains (ZGS 77) - RPAA
B : Experiment : Hornet-UA-1996 combine harvest sample - PMAA
C : Experiment : Hornet-HA-1994 combine harvest sample - PMAA
D : Control : Laboratory sample of 48 hour sprouted grains of Pastiche - PoMS
E : Control : Laboratory sample of 72 hour sprouted grains of Pastiche - PoMS

Schematic representation of the location of *alpha*-amylase activity in gel imprints formed by transverse sections of grains.



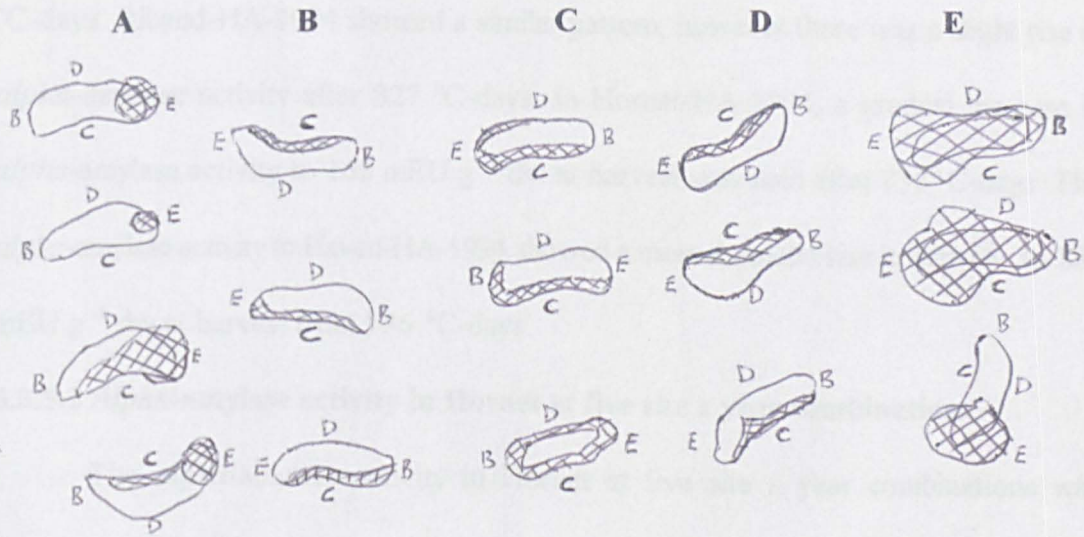
Key
C = crease region, D = dorsal surface, ▨ = *alpha*-amylase activity

Plate 3.3 : *Beta*-limit dextrin gels illustrating location of *alpha*-amylase enzyme activity in longitudinal sections of grains of Hornet from UA in 1996.



Key : A : Control : Laboratory sample of 48 hour sprouted grains of Pastiche - PoMS
B : Experiment : Hornet -UA-1996 combine harvest sample - PMAA
C : Control : Riband-HA-1996 immature grains (ZGS 77) - RPAA
D : Experiment : Hornet -HA-1994 combine harvest sample - PMAA
E : Control : Laboratory sample of 72 hour sprouted grains of Pastiche - PoMS

Schematic representation of the location of *alpha*-amylase activity in gel imprints formed by longitudinal sections of grains.



Key
B = brush region, C =crease region, D = dorsal surface, E = embryo region, ▨ = *alpha*-amylase activity
(See Figure 2.2 for precise location of tissue types)

improve the quantification of this technique, however this would be very time consuming and may still not give the desired level of accuracy. Scanning gels and using image analysis to measure differences in gel staining was attempted, but was not possible due to the poor resolution of the gels.

3.3.5 *Alpha*-amylase activity

3.3.5.1 *Alpha*-amylase activity at HA in 1994

The *alpha*-amylase activity in the grains of the cultivars Hornet, Haven, Pastiche and Riband grown at HA in 1994, from 500 °C days after ear emergence until combine harvesting (1100 °C days) was determined (Figure 3.10). The *alpha*-amylase activity in the samples taken at 500 °C-days was initially high (250-300 mEU g dw) in all four cultivars. The *alpha*-amylase activity in the cultivars then fell and was around 10-60 mEU g^{-1} dw at 730 °C-days. Riband-HA-1994, however, showed a slower decline in *alpha*-amylase activity than the three other cultivars. The *alpha*-amylase activity in Pastiche-HA-1994 then remained relatively constant at around 5-10 mEU g^{-1} dw until harvest at 1051 °C-days. Riband-HA-1994 showed a similar pattern, however there was a slight rise in *alpha*-amylase activity after 827 °C-days. In Hornet-HA-1994, a gradual increase in *alpha*-amylase activity to 162 mEU g^{-1} dw at harvest was seen after 736 °C-days .The *alpha*-amylase activity in Haven-HA-1994 showed a more dramatic rise in activity to 286 mEU g^{-1} dw at harvest from 736 °C-days.

3.3.5.2 *Alpha*-amylase activity in Hornet at five site x year combinations

The *alpha*-amylase activity in Hornet at five site x year combinations was measured and is illustrated in Figure 3.11. A ten to one hundred fold decrease in *alpha*-amylase activity, from 300 °C-days to 700 °C-days, was seen during development at all site x year combinations. There was some disparity between *alpha*-amylase activity during

this initial stage of development, although *alpha*-amylase activity fell at a similar rate at all site x year combinations. This suggests that °C-days after ear emergence may not be an accurate developmental measure for *alpha*-amylase activity. At SB 1994, SB 1995 and HA 1995 *alpha*-amylase activity plateaued out after 800 °C-days at 10-30 mEU g^{-1} dw until harvest, giving rise to a high (336-391 s) HFN (Section 3.3.6). This pattern in *alpha*-amylase activity was not seen at HA in 1994 or 1996, where *alpha*-amylase activity remained around 100 mEU g^{-1} dw after 700° C-days and then showed a slow rise in activity until harvest causing a low (146-273 s) HFN.

3.3.5.3 The relationship between HFN and *alpha*-amylase activity

A negative linear relationship between $\log_{10}$ HFN and $\log_{10}$ *alpha*-amylase activity was identified for flours with an HFN greater than 62 ($\log_{10}$ HFN= (-0.308)$\log_{10}$ *alpha*-amylase activity + 2.89, $P < 0.001$, $R^2 = 0.81$) and is illustrated in Figure 3.12.

3.3.6 Pre-harvest HFN sampling

Table 3.18 shows the pre-harvest HFN (approximately 850 °C-days), and Table 3.19 the combine-harvest HFN for all the site x year combinations studied. At SB 1995 the pre-harvest sample was taken early in development (Section 3.2.10.4), and at UA in 1995 adverse weather (404.8 mm of rain fell between September 1 st and 12 th) led to severe sprouting and prevented both combining of the plots and the recording of harvest HFN.

In 1994 the pre-harvest HFN varied from 154 s (HA - Hornet) to 450 s (SB - Pastiche), giving a broad range of pre-harvest HFN values. At SB all samples had a pre-harvest HFN > 250 s. Samples with a pre-harvest HFN < 250 s were HA - Haven and Hornet, with Riband on the 250 s borderline.

Figure 3.10 : The *alpha*-amylase activity in grains of the cultivars Pastiche, Haven, Hornet and Riband during grain development at HA in 1994

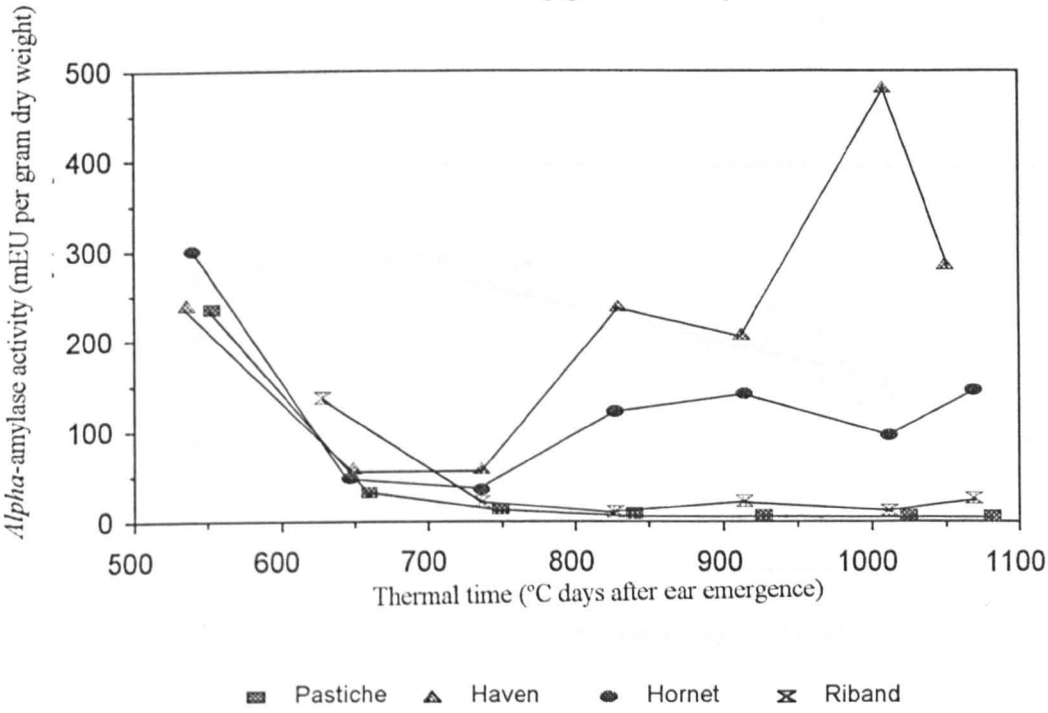


Figure 3.11 : The *alpha*-amylase activity in grains of the cultivar Hornet during grain development at five site x year combinations.

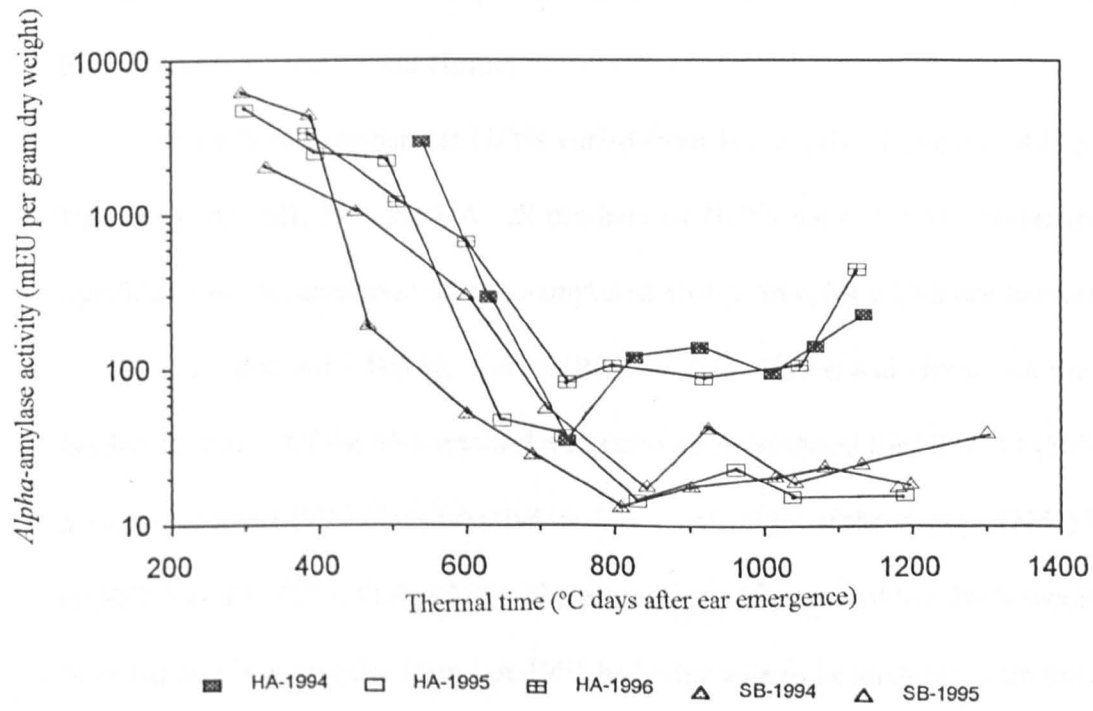
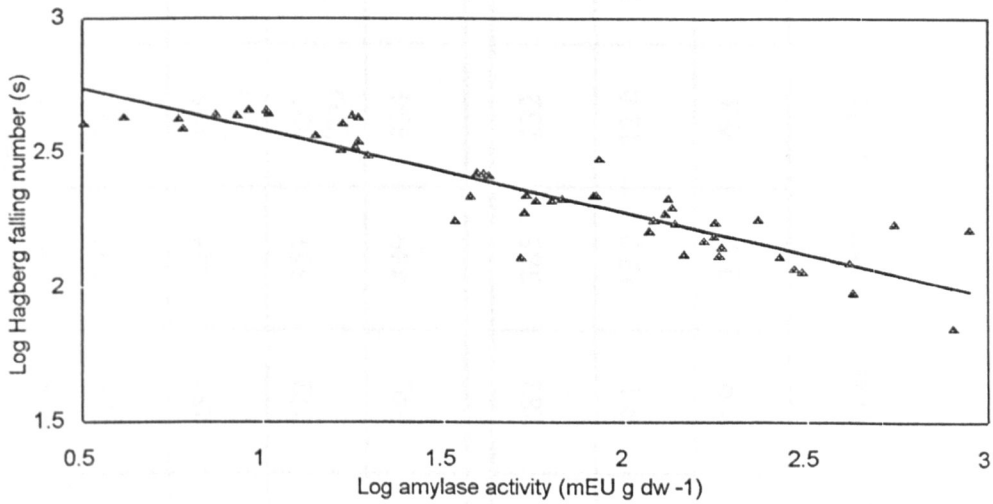


Figure 3.12 : The relationship between Hagberg falling number and *alpha*-amylase activity of flour from combine harvest samples taken at HA and SB 1994.



In 1995 the pre-harvest HFN varied from 189 s (UA - Haven) to 434 s (HA - Pastiche), again giving a broad range of pre-harvest HFN values. At HA all pre-harvest HFNs were >250 s. Samples with pre-harvest HFN < 250 s included AB - Haven, Hornet, Riband and UA - Haven and Hornet.

In 1996 the pre-harvest HFNs varied from 163 s (AB - Haven) to 449 s (SB - Pastiche). At SB, HA and UA all pre-harvest HFNs were > 250 s (illustrating no significant early accumulation of *alpha*-amylase activity). Samples with a pre-harvest HFN < 250 s included AB - Haven, Hornet, Riband, with Haven and Hornet on the 250 s borderline at HA. Of the 36 combined harvested crops analysed for HFN, 14 (39%) had a combine harvest HFN which was below 250 s, with eight of these crops (22%) having an HFN less than 220 s (Table 3.19). The percentage incidences of low HFN would have been higher if the samples from UA 1995 had been able to be analysed, with the severe

Table 3.18 : Mean pre-harvest HFN of UK cultivars taken at 850 °C-days from all field experimental sites in 1994, 1995 and 1996.

Cultivar	1994		1995				1996			
	HA	SB	HA	[1]SB	AB	UA	HA	SB	AB	UA
Haven	173	308	364	380	238	189	258	392	163	278
Hornet	154	409	368	356	234	237	259	348	185 *[2]192*	254 *[2]115*
Riband	268	363	374	369	243	355	272	351	225 *[2]224*	338 *[2]225*
Pastiche	445	450	434	424	350	389	340	449	354	391
Overall Mean	260	382	385	382	268	293	282	385	232	315
SED (6 df)	20.4	26.0	10.2	2.8	20.0	48.2	9.1	12.2	12.0	51.6
CV (%)	9.6	8.3	3.2	0.9	9.1	20.2	3.9	3.9	6.3	20.1

[1] = Sample taken at 470 °C-days. (Section 3.2.11.4), [2] = Fresh samples dried centrally at HA (Section 3.2.11.3).

Table 3.19 : Mean combine-harvest HFN of UK cultivars from all field experimental sites in 1994, 1995 and 1996.

Cultivar	1994		1995				1996			
	HA	SB	HA	SB	AB	[1]UA	HA	SB	AB	UA
Haven	183	327	352	339	227	-	253	239	203	181
Hornet	146	361	391	336	226	-	273	211	207	117
Riband	288	321	376	348	243	-	288	150	245	236
Pastiche	439	447	427	401	345	-	356	357	418	328
Overall Mean	264	364	387	356	260	-	292	239	268	216
SED (6 df)	13.2	23.4	27.0	10.3	9.1	-	18.4	41.0	8.8	20.0
CV (%)	6.1	7.9	8.5	3.5	4.3	-	7.7	21.0	4.0	11.3

[1] = Samples not combined due to severe sprouting.

sprouting seen in these samples leading to the assumption that their HFN would have been around 62 s.

Initial regression analysis on data from HA 1994, showed that pre-harvest HFN and cultivar ($P < 0.001$) had significant effects on combine-HFN and there was also a significant interaction ($P < 0.017$) between pre-harvest HFN x cultivar. A combined regression analysis using data from all the site x year combinations where there was no evidence of sprouting between pre-harvest sampling and combining was therefore carried out for each cultivar separately. Sprouting in samples was identified by visual inspection, FDB analysis, iso-electric focusing and *beta*-limit dextrin assay (Section 3.3.4). For Haven and Hornet, data from four sites, HA 1996, HA 1995, SB 1995 and SB 1994 was used in the analysis. For Riband and Pastiche, data from these four sites and also AB 1996 and HA 1994 was included in the analysis. The following linear relationships between pre-harvest (x) HFN and combine-harvest (y) HFN were identified for each of the cultivars ($y = a + b\,x$) (Table 3.20).

Table 3.20 : Regression equations showing relationship between pre-harvest HFN and combine-harvest HFN for four UK cultivars.

Cultivar	Intercept (a)	Slope (b)	Standard error of the slope	R^2	P
Haven	- 8	1.03	0.214	0.69	< 0.001
Hornet	91	0.68	0.201	0.51	0.008
Riband	76	0.76	0.097	0.79	< 0.001
Pastiche	143	0.66	0.133	0.61	< 0.001

The confidence limits for the target HFN market specifications (y) of 250 s and 225 s were calculated by prediction in reverse i.e. estimating x from y (Snedecor and Cochran, 1973). The 95 % and 75 % confidence limits for the target market HFN

specifications of 225 s and 250 s are shown for the four UK cultivars in Table 3.21.

Table 3.21 : Confidence limits (95 % and 75 %) for the relationship between pre-harvest HFN and combine-harvest HFN for target HFN specifications.

Cultivar	Pre-harvest HFN lower 95 % confidence limit (s)	Pre-harvest HFN lower 75 % confidence limit (s)	Target combine HFN specification (s)	Pre-harvest HFN upper 75 % confidence limit (s)	Pre-harvest HFN upper 95 % confidence limit (s)
Haven	187	211	250	289	313
	158	183	225	267	292
Hornet	115	169	250	331	385
	87	144	225	306	363
Pastiche	161	189	250	311	339
	129	162	225	288	321
Riband	149	196	250	304	351
	120	166	225	284	330

The 95% confidence limits for these relationships were very wide, with the 75% confidence limits being narrower, but still very broad. The 95 % confidence limits for the relationship between the pre-harvest HFN and combine-harvest HFN are illustrated for the four UK cultivars in Figures 3.13-3.16.

3.3.6.1 Relationship between HFN from hand-harvested and combine-harvested samples

Hand-harvested HFN samples tended to give a lower HFN than combine-harvested HFN samples. Linear regression analysis indicated a significant relationship ($y = 0.874\,x + 56.5$, $R^2 = 0.63$, $P < 0.05$) between hand-harvested (x) and combine-harvested (y) HFN (Figure 3.17).

Figure 3.15 : The relationship between pre-harvest and combine harvest HFN of the cultivar Pastiche and the 95% confidence limits at six site x year combinations in the absence of sprouting.

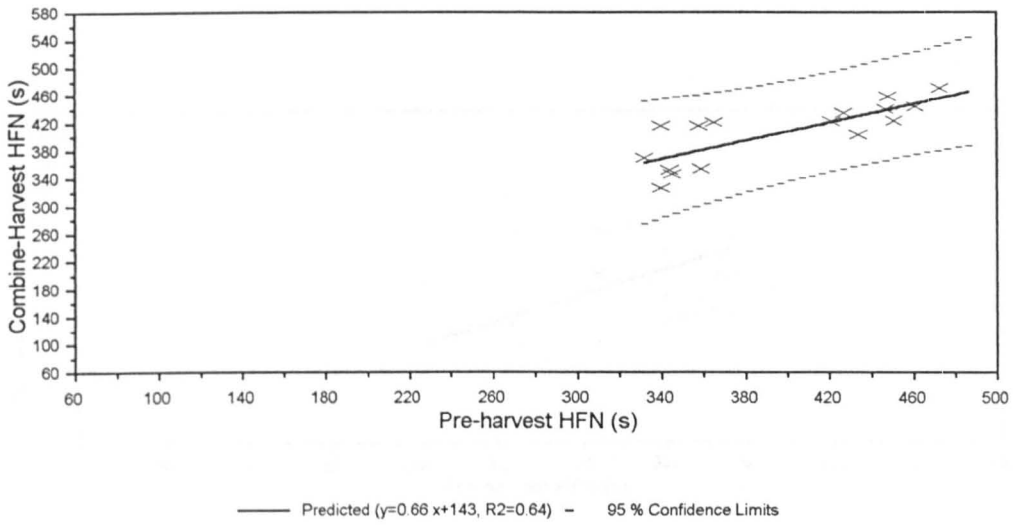


Figure 3.16 : The relationship between pre-harvest and combine harvest HFN of the cultivar Riband and the 95% confidence limits at six site x year combinations in the absence of sprouting.

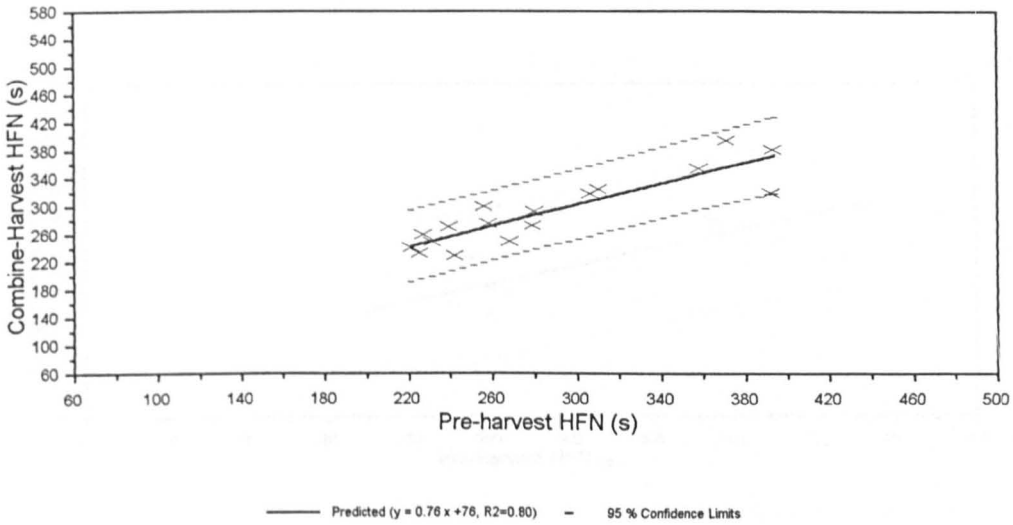


Figure 3.13 : The relationship between pre-harvest and combine harvest HFN of the cultivar Haven and the 95 % confidence limits at four site x year combinations in the absence of sprouting.

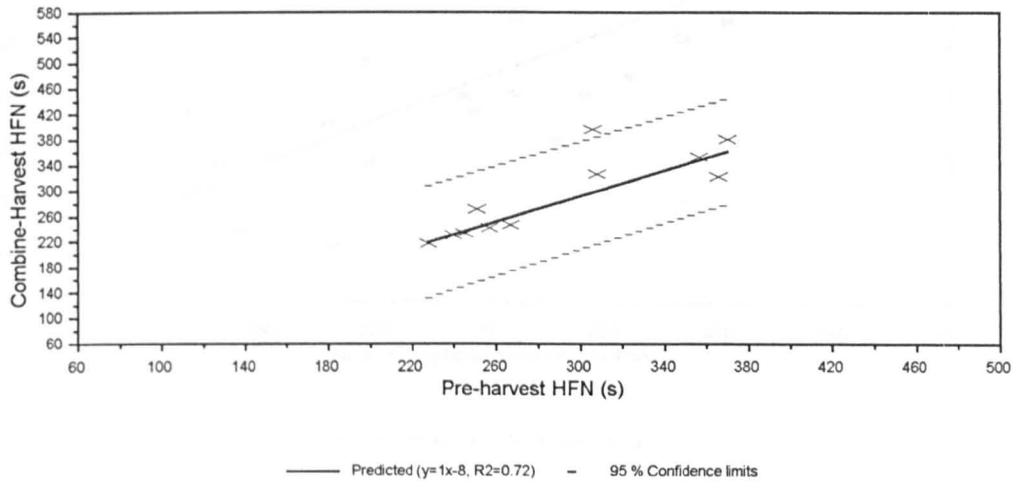


Figure 3.14 : The relationship between pre-harvest and combine harvest HFN of the cultivar Hornet and the 95 % confidence limits at five site x year combinations in the absence of sprouting.

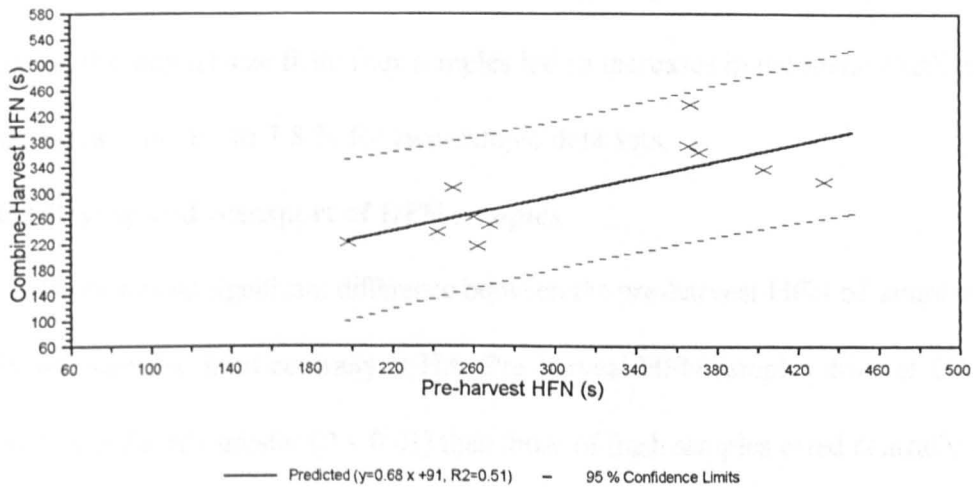
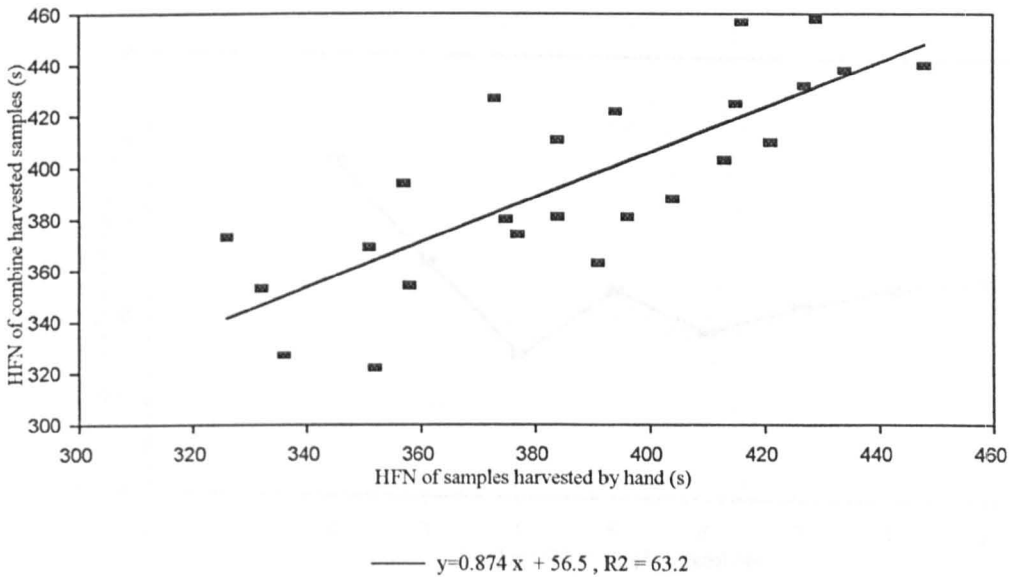


Figure 3.17 : The relationship between the HFN measured from hand-harvested samples and combine-harvested samples at HA in 1995.



3.3.6.2 Precision of hand-harvest HFN sampling

The mean coefficient of variation was calculated for each data set of samples (Figure 3.18). The lowest mean coefficient of variation (5.6%) came from four-sample data sets. Increasing the data set size above four did not lead to any significant improvement in the mean coefficient of variation, which remained around 5.9-6.5%. Decreasing the data set size from four samples led to increases in the mean coefficient of variation, reaching up to 7.8 % for two-sample data sets.

3.3.6.3 Drying and transport of HFN samples

There was no significant difference between the pre-harvest HFN of samples dried at AB and samples dried centrally at HA. Pre-harvest HFN samples dried at UA were however, significantly greater ($P < 0.01$) than those of fresh samples dried centrally at HA (Figure 3.19).

Figure 3.18 : Relationship between the number of pre-harvest HFN samples pooled for HFN analysis and the mean percentage coefficient of variation between the values HFN.

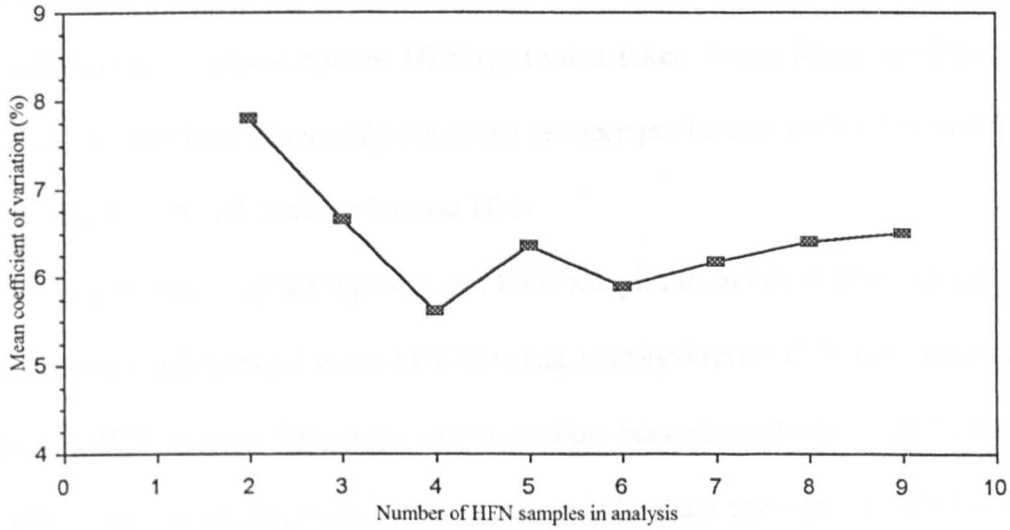
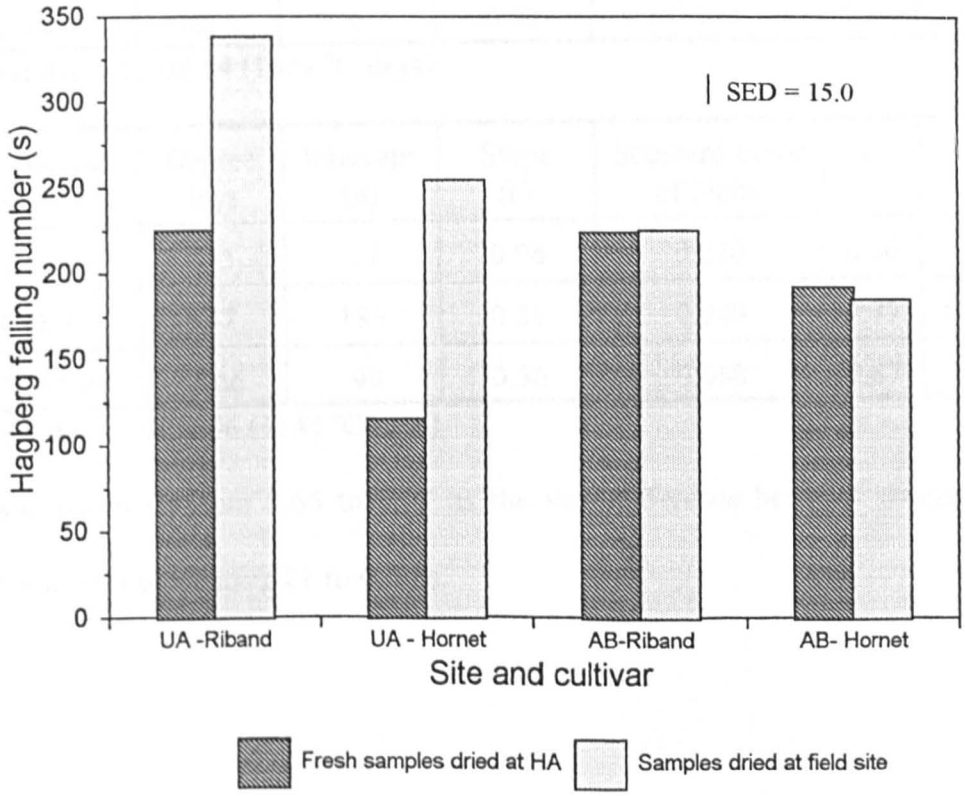


Figure 3.19 : Comparison of pre-harvest HFN samples of Hornet and Riband taken at UA and AB in 1996, and dried either at the field site or transported and dried at HA.



3.3.6.4 Pre-harvest HFN sample time point

Regression analysis of serial pre-harvest HFN samples from HA in 1994, indicated that samples taken 16 days before combining gave a similar relationship between pre-harvest HFN (x) and combine-harvest HFN (y) to that taken 3 days before combining (Table 3.22). No significant relationship was found between pre-harvest HFN taken at 470 °C-days at SB in 1995 and combine-harvest HFN.

Regression analysis of serial pre-harvest HFN samples from HA in 1996, revealed that the relationship between pre-harvest HFN (x) and combine-harvest HFN (y) improved the nearer the HFN sampling time point was to combine-harvesting (Table 3.22). The R^2 statistic improved from 0.66 to 0.87 as the time difference between sampling and combining shortened from 21 to 6 days.

Table 3.22 : Regression equations for the relationship between pre-harvest HFN taken at intervals before combining and combine-harvest HFN at HA in 1994 and 1996.

Pre-harvest Sample Date	Degree days	Intercept (a)	Slope (b)	Standard Error of Slope	R^2	P
03/08/94	827	22	0.93	0.084	0.92	< 0.001
16/08/94	1025	6	0.98	0.118	0.87	< 0.001

Harvest date : 19/08/94 (1069 °C-days)

Pre-harvest Sample Date	Degree days	Intercept (a)	Slope (b)	Standard Error of Slope	R^2	P
12/08/96	815	17	0.98	0.220	0.66	< 0.001
19/08/96	937	136	0.51	0.089	0.77	< 0.001
27/08/96	1066	99	0.56	0.068	0.87	< 0.001

Harvest date : 02/09/96 (1144 °C-days)

3.4. Discussion

3.4.1 Weather data

Variations in the weather at the ten site x year combinations gave a representative spread of conditions likely to be experienced by UK wheat crops in the field. Unfortunately the weather conditions did not stimulate the wide occurrence of PMAA as occurred in the UK in 1985 (Flintham and Gale, 1988), thus restricting the size of the data set for analysis of factors stimulating PMAA. Only two of the 40 possible site x year x cultivar combinations showed solely the occurrence of PMAA (5% of the total possible combinations), with PMAA occurring in a further eight cases alongside PoMS (20% of the total possible combinations) and in one instance alongside RPAA in Riband at HA 1994 (2% of the total possible combinations).

3.4.2 Plant development

The relative rates at which the UK cultivars reached harvest ripeness reflected their NIAB ratings for earliness of ripening (Anon., 1991). The use of °C-days after ear emergence as a marker for grain development was not as reliable as anticipated with quite large variations occurring between accumulated temperature and grain growth stage at the ten site x year combinations. Whether this was due to the natural heterogeneity of the field crops is unclear. The use of °C-days after ear emergence as a sampling time point in these field experiments was largely employed to examine the relationship between grain dormancy and temperature (Gate, 1995). Basing the accumulated temperature from the start of anthesis may have been more appropriate in giving a standard grain developmental timescale as this is when fertilisation of the grain occurs and the genetic make-up of the grain is complete (Section 2.2.2.1) . Using a base temperature of 9 °C from anthesis may also have aided in standardising the grain developmental timescale as has been used in

other studies (Weir *et al.*, 1984).

3.4.3 Moisture content and grain drying-rate

The grain drying-rates obtained in the multi-site field experiment ranged from 1.14 to 3.41 % moisture loss day $^{-1}$ illustrating the wide range of drying rates cultivars may experience in the field. The upper figure of 3.41 % moisture loss day $^{-1}$ is rather high compared to other field studies, where grain drying-rates have been measured between 0.85-2.6 % moisture loss day $^{-1}$ (Gold, 1991) and 0.44-2.07 % moisture loss day^{-1} (Kettlewell and Cashman, 1997). They are, however, consistent with the inclusion of data from the very dry year of 1995, with other studies in that year also showing grain drying-rates greater than 3 % moisture loss day $^{-1}$ (Kettlewell, 1998). Overall the years could be ranked in the order of fastest grain drying-rates as 1995 > 1994 > 1996, with sites ranked in the order SB > HA > AB > UA, largely reflecting the temperatures experienced during the dough stage of grain development, with high temperatures causing a faster grain drying- rate as expected. There was little difference in the overall grain drying-rates of the cultivars with Haven having a slightly faster grain drying-rate than Riband and Hornet, with Pastiche having the slowest grain drying-rate. This overall ordering of cultivars in relation to grain drying-rate did not reflect their overall ordering in respect to combine harvest HFN (Pastiche >Riband > Hornet > Haven). This was probably due to the confounding effects of PoMS, with the combine HFN of Haven very susceptible to PoMS, hence giving low HFN values unrelated to PMAA.

The occurrence of PoMS alongside PMAA hindered a full investigation into the relationship between grain drying-rate, HFN and PMAA, but overall slower grain drying rates were shown to be linked with the occurrence of PMAA. Grain drying-rates of 1.92 % moisture loss day $^{-1}$ (Hornet, HA 1994) and 1.79 % moisture loss day $^{-1}$ (Riband, AB

1995) were shown to be correlated with high PMAA. These rates are greater than those identified by Gale *et al.* (1983) as causing high PMAA, and greater than the 1.07 % moisture loss day $^{-1}$ proposed by Gold and Duffus (1995) to suppress PMAA. This variation in apparent sensitivity to grain drying-rate may be due to cultivar differences between studies or due to the measurement method used to derive grain drying-rate estimates. Gold (1991) used linear regression analysis to estimate grain drying-rates of cultivars from field experiments, and the grain drying- rates of the cultivars in the controlled-environment cabinet studies of Gale *et al.* (1983) have also been estimated by linear regression. Linear functions may not however be the best functions to fit to derive estimates of grain drying rates between 40-20 % grain moisture content. For example, only 59 % of the variation in percentage moisture content over time was explained by linear regression analysis in some of the treatments applied by Gold (1991) in field experiments. This compares to values of, for example, 82.2 % of the variation accounted for using linear regression analysis in this current study. Second degree polynomial curves (Dodds and Pelton, 1967) and logistic curves (Kettlewell and Cashman, 1997) have all been applied to grain moisture data to try and derive more accurate estimates of grain drying-rates. With the accuracy of linear regression estimates of grain drying-rate varying between studies, it is perhaps not surprising that a clear quantitative relationship between grain drying-rate and PMAA has failed to emerge between studies. However, the difference between the slow drying rate of Hornet (HA 1994) where PMAA was detected and fast drying rate of Hornet (HA 1995) where no PMAA was detected was 1.13 % moisture loss day^{-1}, similar to the difference (1.05 % moisture loss day^{-1}) in fast and slow grain drying-rates shown to previously cause differences in PMAA (Gale *et al.*, 1983). This therefore supports the hypothesis that differences in PMAA can be mediated by

different grain drying-rates (Gale *et al.*, 1983). In contrast, only a small difference (0.1 % moisture loss day $^{-1}$) was seen between the grain drying-rate of Hornet (HA 1994) and Hornet (SB 1995), even though there was a large difference in HFN (190 s), supporting the hypothesis that other factors also influence PMAA (Gold and Duffus, 1996).

Other recent studies have also failed to establish a clear relationship between grain drying-rate and HFN or *alpha*-amylase activity (Kettlewell and Cashman, 1997). Although conversely significant relationships between cumulative potential evapotranspiration calculated over certain periods during grain ripening and HFN have been identified (Kettlewell, 1997), seeming to support the role of grain drying-rate in enhancing PMAA. A possible reason for the above discrepancies is that in some instances the cultivars were not all sampled on exactly the same calendar day, thus weather conditions experienced during grain drying may have been slightly different and thus masked any relationships (Clarke, 1983).

The results from these experiments do not provide conclusive evidence that slow grain drying-rates stimulate enhanced PMAA in the grain although they do add support to this hypothesis (Gale *et al.*, 1983). The use of grain drying-rate to predict combine HFN is, however, of limited practical use to growers as it is measured retrospectively. The time available for growers to make decisions based on predictions from monitoring grain drying-rate would be very small (Kettlewell, 1993). Relating grain drying-rate to actual environmental conditions and their effects on grain drying may however enable more timely predictions to be made.

It has been suggested that another environmental factor associated with seasonal differences in grain drying-rate and cumulative potential evapotranspiration is responsible for enhancing PMAA in the grain (Kettlewell and Cashman, 1997). The identification of

the possible environmental factor causing enhanced PMAA in the grain is further investigated in Section 4.

3.4.3.1 Comparison of grain drying treatments

The relationship identified is similar to a previous comparison between freeze-drying (y) and oven-drying (x) where $y = 1.034x - 3.935$ (Astbury and Kettlewell, 1991) and also reflects a comparison made by Gold (1991), who showed that oven-drying gave slightly higher moisture content (%) values than freeze-drying. The drying methods are therefore not precisely comparable, but they do enable relative comparisons between the drying rates of samples to be made.

3.4.4 Source of *alpha*-amylase in combine harvest samples

Table 3.23 illustrates the assigned source of detectable *alpha*-amylase activity in the four cultivars at the experimental sites. Of the 40 crops analysed, 22 cases (55%) showed some detectable *alpha*-amylase activity. The allocation of the source of *alpha*-amylase activity in these samples is based on the combined results from Tables 3.16 and 3.17.

The presence of visual sprouting clearly indicated PoMS had occurred in the samples in which it was seen. Visual sprouting was evident in all cultivars at UA-1995, and in all cultivars except Pastiche at UA-1996 and SB-1996, and in Haven at HA-1994. Furthermore, evidence of FDB staining indicated PoMS had occurred in Haven, Hornet and Riband at HA-1996 and in Haven-AB-1995. Contamination of the harvest sample of Hornet-HA-1994 with a few grains of Recital caused an initial positive FDB result. On analysing a sample where the grains of Recital had been removed no FDB staining was seen, thus PoMS was not attributed as the cause of low HFN in Hornet-HA-1994. The *beta*-limit dextrin gels indicated *alpha*-amylase activity in the embryo region of grains of

Table 3.23 : Source of detectable *alpha*-amylase activity in four cultivars from four UK field experimental sites between 1994 and 1996.

Cultivar	1994		1995				1996			
	HA	SB	HA	SB	AB	UA	HA	SB	AB	UA
Haven	***PoMS***	[2]-	[1]-	[1]-	***PoMS*** + ***PMAA***	***PoMS***	PoMS	***PoMS***	***PoMS*** + ***PMAA***	***PoMS*** + ***PMAA***
Hornet	***PMAA***	[2]-	[1]-	[1]-	***PMAA*** + ***PoMS***	***PoMS***	PoMS	***PoMS***	***PoMS*** + ***PMAA***	***PoMS*** + ***PMAA***
Riband	RPAA + PMAA	[2]-	[1]-	[1]-	***PMAA***	***PoMS***	PoMS	***PoMS***	***PoMS*** + ***PMAA***	***PoMS*** + ***PMAA***
Pastiche	[1]-	[2]-	[1]-	[1]-	[1]-	***PoMS***	[1]-	[1]-	[1]-	[2]-

HFN less than 250 s

HFN greater than. > 250 s

[1]- *Alpha*-amylase activity too low for detection

[2]- Sample not available for *beta*-limit dextrin gel test, but most probably *alpha*-amylase activity too low for detection

No cases of : RPAA (Retention of pericarp *alpha*-amylase activity) or PrMS (Pre-maturity sprouting) caused the combine-harvest HFN to fall below 250 s.

Haven, Hornet and Riband at AB-1996 and in Hornet-AB-1996 demonstrating PoMS had also occurred in these samples. PoMS was, thus, attributed to 19 of the combine harvest samples.

The results from the *beta*-limit dextrin gels and IEF gels allowed the occurrence of PMAA to be assigned to the samples. The identification of *alpha*-amylase activity in the crease region of the grain allowed PMAA to be assigned to Hornet-HA-1994, Hornet-AB-1995, Riband-AB-1995, and in Haven, Riband and Hornet at both AB 1996 and UA 1996. The absence of grains from Haven-AB-1995 prevented a beta-limit dextrin gel test being undertaken, it cannot, therefore, be ruled out that only PoMS is occurring in this sample. However, the occurrence of only α-AMY-1 bands on the IEF gel and a low pre-harvest HFN (238 s) strengthens support for the occurrence of PMAA in Haven-AB-1995. Similarly Hornet-HA-1994, had a low pre-harvest HFN (154 s) and IEF analysis of hand-harvested grains identified only α-AMY-1 bands, suggesting PMAA was the cause of a low HFN. Thus, the occurrence of PMAA was identified in 10 of the cases. The only case in which RPAA was identified was Riband-HA-1994, with faint α-AMY-2 bands identified by IEF.

Of the 40 crops analysed, 18 cases (45%) showed no detectable *alpha*-amylase activity, 11 cases (28%) showed PoMS only, 8 cases (20%) showed both PoMS and PMAA, 2 cases (5%) showed PMAA only and 1 case showed both RPAA and PMAA

Overall the cultivars could be ranked in order of highest combine HFN as Pastiche > Riband > Hornet > Haven which is as would be expected from their NIAB ratings (Anon, 1991) and is the same order as has been found in other studies including these cultivars (Smith and Gooding, 1999). The principal cause of the combine HFN falling below 250 s was identified as PoMS, which occurred at 16 out of 18 site x year

combinations (89% of these cases). PMAA was identified as a sole cause of HFN falling below 250 s at two site x year combinations and a contributory cause at a further eight site x year combinations. Thus, 11 % of cases where the combine HFN fell below 250 s was primarily due to PMAA, with it also playing a role with PoMS in a further 44 % of cases.

Neither PrMS or RPAA were identified as a principal cause of low HFN at any of the site x year combinations. It should however be noted that low amounts (< 1 % of grains) of visual WOBM damage were seen in grains at HA in 1994, although no evidence of PrMS was seen. WOBM damage was also identified at very low levels at most site x year combinations but with no evidence of PrMS. Similarly RPAA did not cause a fall in HFN to below 250 s in any of the cases studied. At HA in 1994 there was evidence of RPAA in grains of the cultivar Riband but this was not sufficient to decrease the HFN to below 250 s. PMAA was identified in three of the four UK cultivars tested, Haven, Hornet and Riband, but was not identified in Pastiche. This suggests that expression of PMAA may be inherent in many of the commercial cultivars currently grown in the UK and may be the reason for sporadic instances of low HFN in some cultivars e.g. Rialto in 1998 (Anon., 1998). This supports the suggestion that the occurrence of PMAA is more of a problem than generally appreciated (Gale, 1989). PoMS occurred more so in Haven than the other cultivars, with Hornet showing similar or greater amounts of PoMS than Riband, with Pastiche showing the least PoMS. This reflects the relative sprouting susceptibility scores attributed to them by NIAB (Anon., 1991). These results thus seem to support the method used by NIAB to assess the sprouting resistance of cultivars (McVittie and Draper, 1982a). It should, however, be borne in mind that the cultivars selected in this experiment were from broad ends of the "sprouting scale " and differentiating between cultivars with similar resistance may not be so accurate.

These results are the first quantification of the actual occurrence of the four sources of *alpha*-amylase which can cause a low HFN in wheat crops and highlight their relative importance. The results also demonstrate that ascribing a single route as a cause of low HFN in a crop may often be erroneous with several routes possibly interacting and causing an accumulation of high *alpha*-amylase activity and low HFN. Some caution should however be applied to these interpretations as α-AMY-2 isoenzymes are normally identified in germinating grain after the appearance of α-AMY-1 isoenzymes (Gale and Ainsworth, 1984). It is thus possible that some cases classified as PMAA may in fact be the early stages of PoMS. Using the *beta*-limit dextrin and Phadebas gels helps overcome this problem to some extent by locating the areas of *alpha*-amylase activity in the grain, but this technique still does not allow the cause of high *alpha*-amylase activity to be assigned with 100 % confidence in all cases. This is due to the fact that *alpha*-amylase activity is seen along the crease region of the grain as PoMS progresses (Cornford *et al.*, 1987b).

3.4.5 *Alpha*-amylase activity

Duplicate measurements made on the same sample extract rarely fell outside the ±5% range of acceptability. More variation was apparent in the extraction of *alpha*-amylase activity from duplicate flour samples. This can be attributed to the inherent variability in the *alpha*-amylase activity within a flour sample (McVittie and Draper, 1982b :Vaidanyathan, 1987). The effect of this inherent background variability must be borne in mind when considering the results.

3.4.5.1 *Alpha*-amylase activity at HA in 1994

The high initial *alpha*-amylase activity recorded in the grains was shown, by IEF, to be due to the presence of α-AMY-2 isoenzymes, commonly known to be located in

the pericarp (Olered and Jonsson, 1970). The decline in *alpha*-amylase activity as the grain developed was probably due to the degradation of α-AMY-2 activity in the pericarp of the grain (Olered and Jonsson, 1970). The slower fall in this activity in Riband may account for the low HFN recorded in this cultivar at harvest. The low activity in Pastiche at harvest reflects its high sprouting resistance and very high HFN rating (Anon., 1991). The rise in *alpha*-amylase activity in Haven, could be accounted for by the slight sprouting induced by the showery weather before harvest. In Hornet,the rise in *alpha*-amylase activity corresponded to a grain moisture content of 47.8 % (Section 3.3.3.2) . In other cultivars susceptible to high PMAA the increase in *alpha*-amylase activity occurred at a slightly lower grain moisture content. In Maris Huntsman, *alpha*-amylase activity began to increase when the grain moisture content reached approximately 45 % (Gale *et al.*, 1983) and a similar rise in *alpha*-amylase activity in Fenman began at a moisture content of 41 % (Cornford and Black, 1985).

The fact that *alpha*-amylase activity around 850 °C-days corresponded to that at harvest ripeness suggests that a pre-harvest HFN sampling time point of 850 °C-days was appropriate in this year (Section 3.3.6.).

3.4.5.2. *Alpha*-amylase activity in the cultivar Hornet at five site x year combinations

The plateauing out of *alpha*-amylase activity around 800 °C-days suggests this is the stage in grain development where pericarp (α-AMY-2) amylase is finally degraded, although *alpha*-amylase activity does seem to persist at low levels. The rise in *alpha*-amylase activity in Hornet at HA 1994 and HA 1996 both occurred around 700 °C-days, well before the first sampling time point for pre-harvest HFN at 850 °C-days. This illustrates that sampling at this stage in development is early enough to detect PMAA in

most cases. The subsequent rise in *alpha*-amylase activity after 850 °C-days may account for the large reduction seen between pre-harvest HFN and combine HFN in some cases (Section 3.3.6.1). The difference in *alpha*-amylase activity at the site x year combinations illustrates the dominant effects of the environment on individual cultivars susceptible to high PMAA. The identification of environmental factors causing these differences is considered in Section 4.

3.4.5.3 The relationship between HFN and *alpha*-amylase activity

This relationship is comparable to that identified by Moot and Every (1990). This relationship can be used to infer the HFN in a flour sample from an *alpha*-amylase activity measurement. The accuracy of this prediction will however be adversely affected by the starch properties of the grain, particularly when *alpha*-amylase activity is low (Corr and Spillane, 1969 ; Ringlund, 1983).

3.4.6 Pre-harvest HFN sampling

The linear relationships identified between pre-harvest and combine-harvest HFN illustrate that pre-harvest sampling can be used to give an early indication of the likely combine HFN of a crop if weather conditions remain favourable to harvest and PoMS is not induced. The relationships however had very broad 95 % confidence limits, but are similar to those found in previous attempts to develop an HFN prediction model (Teittinen *et al.*, 1994). It is important that the accuracy of these relationships is improved as the critical commercial decision point occurs in the HFN range 225-250 s. In assessing the accuracy of the 40 pre-harvest HFN predictions, only 36 combine harvest HFN samples were able to be determined. If growers wished to be sure the HFN of their crop was greater or less than 250 s only 17 out of the 36 samples enabled a prediction with 95 % confidence to be made. The other 19 pre-harvest HFN samples all fell in the

uncertain area. Similarly if growers wished to be sure the HFN of their crop was greater or less than 225 s, only 19 out of the 36 pre-harvest samples enabled a prediction with 95 % confidence to be made. The use of 75 % confidence limits narrowed the prediction limits but they were still broad. If growers wished to be sure the HFN of their crop was greater or less than 250 s, only 23 out of the 36 pre-harvest samples enabled a prediction with 75 % confidence to be made, with similar accuracy seen at 225 s specification. Clearly there is need for improvements in these predictions, if an accurate quantitative pre-harvest HFN prediction is to be made. Coupling the use of pre-harvest HFN results with grain drying-rate data may improve the accuracy of the predictions, by accounting for changes in PMAA between pre-harvest HFN sampling and combine-harvesting.

3.4.6.1 Relationship between the HFN of hand- and combine-harvested samples

The significant relationship between the HFN of hand- and combine-harvested samples validates the hand-sampling approach for estimation of combine-HFN. The R^2 statistic however is not as good as may have been expected. This may be due to all the HFN values lying in the higher region of the HFN scale between 300-460 s where starch properties of the grain begin to have a significant effect on HFN (Section 2.1.5). The slightly lower HFN values recorded for the hand-harvested sample may be due to the retention of grains which are normally lost in the mechanical process of combine-harvesting. Larger grains would be expected to be retained in combining with smaller grains lost (Hall, 1991). Evers *et al.*(1995) suggested that grain size is related to *alpha*-amylase activity, with larger grains possessing higher *alpha*-amylase activity. This suggestion however does not explain the difference between the samples as the combine-harvest samples had a higher, rather than lower HFN than the hand-harvested HFN samples.

The possibility that sample size is affecting the results must also be considered. Combine-samples consisted of 1.5-3.3 kg of grain whereas hand-samples consisted of 250-350 g of grain. Errors during sampling may have effected the values of HFN, although a consistently lower trend as occurred would not be expected (Tipples, 1971). The reasons for differences between hand-HFN and combine-HFN therefore remain unclear.

3.4.6.2 Precision of hand-harvest HFN sampling

The results demonstrated that taking fewer than four samples, increased the coefficient of variation, leading to a less precise pre-harvest HFN. Increasing the number of samples above four did not dramatically improve the precision of the measurement. Sampling for pre-harvest HFN should therefore be based on pooling not fewer than four sample points from within each plot to obtain a representative sample of 300-350 ears for HFN determination. A degree of caution should however be attached to these results as sampling along the length of the plots was carried out systematically rather than randomly within the plots (Bloom, 1985). Whether this sampling system would be equally applicable to large commercial fields is unclear.

3.4.6.3 Drying and transport of HFN samples

The variation between the pre-harvest HFN of samples grown at UA, and then dried at UA or transported to HA and then dried , suggests either:- a) excessive drying temperatures (> 50 °C) may have been used at UA leading to some inactivation of *alpha*-amylase activity, or, b) transport of samples is allowing the accumulation of *alpha*-amylase activity between sampling and analysis. The low combine-harvest HFN recorded at UA suggests that a) is the more likely cause. These results indicate the importance of maintaining drying conditions < 50 °C during preparation of pre-harvest HFN samples for

threshing and milling. The centralised drying of samples, where conditions could be closely monitored, would therefore appear to be the most prudent option in a practical pre-harvest HFN sampling prediction scheme.

3.4.6.4 Pre-harvest HFN sampling time point

In 1994 at HA the relationship between pre-harvest and combine-harvest HFN was very similar for samples taken three and sixteen days before harvest, indicating little variation in the effect of environmental factors on HFN during this time period.

The lack of relationship between pre-harvest HFN at 470 °C-days taken at SB in 1995 and combine-harvest HFN is not unsurprising, due to the fact that the grain had not completed grain filling at this stage in development and still possessed visible green colour. The persistence of pericarp α-AMY-2 would have been expected to produce low pre-harvest HFN, although the high pre-harvest HFN values recorded, suggest that this may not be the case in this instance. It is possible that the drying regime employed for these samples inactivated the pericarp α-AMY-2 in the pre-harvest sample. Starch properties of the grain as well as *alpha*-amylase activity are also important in determining HFN in the upper regions of the HFN scale (Corr and Spillane, 1969 ; Ringlund, 1983). Samples taken at 470 °C-days have not completed full development of the starchy endosperm (Simmonds and O'Brien, 1981) and thus are not likely to reflect combine-harvest HFN.

In 1996 the relationship between pre-harvest HFN and combine-harvest HFN at HA improved the nearer pre-harvest sampling time point was to harvesting, as expected, as environmental factors had less time to exert an influence on HFN. The time taken to process samples and generate results must give growers approximately 7-10 days advance notice for effective management decisions to be made in the hectic harvesting period.

These results show that it is possible to predict HFN 10-14 days in advance of combining, by taking a pre-harvest HFN sample at 850° C days after ear emergence (35 % moisture / ZGS 85-87) thus enabling a grower time to make practical use of the results.

3.5 General Conclusion

The field experimental work has allowed the testing of a variety of techniques enabling the route causing high *alpha*-amylase activity in field harvested grain to be established. This has allowed the cause of low HFN to be assigned to a specific route, although in some instances this was still not equivocal. It has been possible to assign a quantitative measure to the relative occurrence and importance of PMAA, with two cases out of 18 (11%) in which the HFN fell below 250 s attributable solely to PMAA. This is a relatively high percentage of cases and illustrates the importance of high PMAA to UK wheat quality. Three out of the four UK cultivars tested showed susceptibility to high PMAA in the field during the course of the ten site x year investigations. This suggests that the phenomenon of PMAA is probably quite common in commercial UK cultivars. This perhaps not unsurprising considering that the cultivars possess a similar genetic background (Table 3.3), with the cultivar Norman being a descendant of Hobbit which has the PMAA susceptible cultivar, Professeur Marchal in its pedigree (Jarman *et al.*, 1993).

The occurrence of PMAA was often linked with PoMS, 44 % of cases where the HFN fell below 250 s. It is thus possible that PMAA may be promoting the more rapid development of PoMS symptoms once dormancy is broken (Gale *et al.*, 1983).

PMAA was first seen in the grain at around 700 °C-days, and was certainly present in the grain when pre-harvest HFN samples were taken at 850 °C-days. A subsequent rise in PMAA after 850 °C-days may explain some of the inaccuracies in the pre-harvest HFN

predictions made and the wide confidence limits obtained.

Pre-harvest HFN sampling at 800-850 °C-days gives an indication of the likely harvest HFN of the crop, in the absence of subsequent adverse weather conditions. Crops with a pre-harvest HFN lying outside the lower 95 % confidence limits of the prediction are unlikely to reach target HFN specifications. Growers can therefore focus their attention on crops whose quality is still likely to meet target specifications. This would enable better judgement to be applied to harvesting and the drying priority of individual crops, and ensure the quality of the crops harvested is optimised despite the prevailing weather. Further quantification of the effect of weather factors on *alpha*-amylase activity between sampling and harvesting is necessary to improve the confidence limits around the prediction. The precise timing of this sample needs to be refined, however, as there is evidence that pericarp (α-AMY-2) amylase may be persisting in the grain at this current sampling point. The imprecise matching between °C-days after ear emergence (base temperature 0 °C) and *alpha*-amylase activity profiles suggests the use of some other plant developmental timescale may allow a more precise sampling time point to be specified with moisture content seeming a more appropriate timescale to use.

4. Controlled-Environment Cabinet Experiments Investigating the Effects of Transient Temperature Changes on Pre-Maturity *Alpha*-Amylase Activity (PMAA)

4.1 Introduction

The induction of high PMAA in grains is highly dependent on the environment (Section 2.7.3). Little attention has been paid to identifying and quantifying the precise effects of environmental factors on PMAA in the grain. Any environmental factor which adversely affects the control mechanisms regulating *alpha*-amylase production in developing grains, may be responsible for the generation of high PMAA (Cornford *et al.*, 1987).

The *alpha*-amylase activity in cultivars susceptible to high PMAA generally starts to rise as the moisture content of the grain falls from 40-20%. The moisture content (%) of the grain declines progressively during grain growth, and until the beginning of the dough stage, at around 40% grain moisture content, this occurs by increases in dry weight with little change in absolute water content of the grain (Meredith and Jenkins, 1975). Subsequently during the dough stage the grain loses water at a rate dependent on temperature and humidity. Initial studies using low and high relative humidity in controlled-environment cabinets to generate fast (1.85% moisture loss day $^{-1}$) and slow (0.80% moisture loss day^{-1}) grain drying-rates between 40-20% grain moisture content, illustrated that slow grain drying enhanced PMAA (Gale *et al.*,1983). This effect was also seen in the field experiments (Section 3).

Environmental conditions such as cold temperatures, rainfall and high humidity that delay senescence by inhibiting grain drying have been proposed as the principal factors determining PMAA in harvest ripe grain (Flintham and Gale, 1988). Other field experiments have implicated low temperature, low hours of sunshine and dew as factors

inhibiting grain desiccation and increasing PMAA in the grain (Matsuzaki and Toyoda, 1996). Field experiments monitoring the effects of grain drying-rate on *alpha*-amylase activity, have however, failed to identify a clear relationship between the two (Section 2.7.3.1). This implies that some other factor apart from grain drying-rate may be responsible for affecting PMAA in the grain. The nature of this factor is currently unidentified.

A possible indicator of the environmental factor causing this effect comes from several studies in northern hemisphere glasshouses. These studies have revealed higher PMAA in glasshouse grown plants as opposed to field grown plants (Bingham and Whitmore, 1966 ; Evers and Ferguson, 1980 ; Gold, 1991). Characteristic features of the glasshouse environment, such as, periods of high humidity, high temperature and large diurnal ranges in temperature, have been suggested as factors acting as a possible stimulus for high PMAA (Gold, 1991). It was proposed that one or more factors may stimulate a certain number of grains to start producing *alpha*-amylase with grain drying-rate determining the degree of PMAA in these grains. It was hypothesised that increased aleurone responsiveness to GA, at higher moisture contents, would increase the time period in which the grain possessed sufficient moisture content for *alpha*-amylase synthesis. This would lead to the observed increase in *alpha*-amylase activity. If aleurone responsiveness is triggered at later stages in development, at lower moisture contents, there may be insufficient moisture availability for *alpha*-amylase synthesis. Moisture content of the grain and drying conditions would thus modulate the actual amount of *alpha*-amylase synthesis that occurs (Gold and Duffus, 1996).

Identification and quantification of the effects of environmental variables on PMAA would be beneficial in the development of an agrometeorological model for

preharvest prediction of HFN (Kettlewell *et al.*,1996), particularly, as any *alpha*-amylase synthesised during grain maturation may lead to the more rapid development of pre-harvest sprouting symptoms following dormancy break (Gale *et al.*, 1983). The identification of environmental variables causing high PMAA would also aid plant breeders in developing a screen to identify cultivars susceptible to the trait.

Five controlled-environment cabinet experiments were undertaken to study the effect of specific transient changes in temperature, at different stages in grain development, from anthesis to harvest ripeness, on PMAA in the grain. The effects of transient changes in temperature, rather than prolonged changes in temperature were undertaken to try and identify specific stages in grain development which may be susceptible to the stimulation of PMAA. The overall objective of these experiments was to establish whether temperature plays a role in stimulating PMAA before physiological maturity, as opposed to its effects after physiological maturity when high temperature could influence grain drying-rate and exert effects on moisture availability in the grain.

Experiment 1 investigated the hypothesis that a period of high temperature before physiological maturity could stimulate high PMAA in the grain. The effect on *alpha*-amylase activity caused by transferring plants from a cool to a hot temperature regime, for a 72 hour period, at one of four stages in grain development, was investigated.

Experiment 2 investigated the hypothesis that a period of high temperature at earlier stages in grain development than those examined Experiment 1, could stimulate high PMAA in the grain. Plants were transferred from a cool to a hot temperature regime for a 72 hour period at one of four stages in grain development, including similar stages to Experiment 1, to check the repeatability of the results from Experiment 1.

Experiment 3 was undertaken to investigate the hypothesis that it was a specific

transient change to a high temperature that was responsible for causing changes in *alpha*-amylase activity, rather than a temperature change *per se* that stimulated high PMAA. To investigate this, plants were reciprocally transferred between cool and hot temperature regimes at grain development stages shown to be susceptible to the generation of high PMAA in Experiments 1 and 2. Transfer at only one stage in grain development was possible, with a temperature fault in the cabinet with the hot temperature regime preventing attempts to transfer plants at other grain developmental stages.

Experiment 4 again investigated the hypothesis that large transient increases or decreases in temperature during grain development could stimulate high PMAA. Reciprocal transfers of plants between cool and hot temperature regimes at three different stages during grain development were undertaken to identify their effect on PMAA.

Experiment 5 investigated the hypothesis that PMAA in the grain would be affected by variations in the length of time the plants were exposed to a transient temperature change. Time of reciprocal transfer between the cool and hot temperature regimes was increased to 120 hours and 240 hours, with transfers being undertaken at one of two stages during grain development, with grain developmental stages selected on the basis of the results from the previous controlled-environment cabinet experiments. This experiment was an attempt to try and quantify the effect of duration of temperature change on PMAA.

4.2 Materials and methods

4.2.1 Plant husbandry

Dressed (Rappor® a.i.= guazatine) C2 trials seed of three cultivars of winter wheat, (*Triticum aestivum* cv. Pastiche, Riband and Hornet) used in the field experiments

(Table 3.3), were also used in the controlled-environment cabinet experiments. It was hoped that this would assist in comparisons between the field results (Section 3.3) and the controlled-environment cabinet experiment results. The cultivars Pastiche and Riband were used in Experiments 1 and 2. Pastiche was used as it has high sprouting resistance rating and HFN (Anon., 1991) and was thus presumed to have a low propensity to produce PMAA and would thus act as a control in experiments. Riband was included as it is currently widely grown commercially, is relatively sprouting resistant, but has only a medium HFN value and was, therefore, assumed to be susceptible to the occurrence of PMAA (Anon.,1991). Results generated from this cultivar were thus more likely to be of practical use to growers in an HFN prediction scheme (Section 1). Hornet, assumed to be the most susceptible cultivar to PMAA, due to it possessing a low HFN rating, but being relatively sprouting resistant, was not initially included in experiments, due to concerns over its susceptibility to mildew (*Erysiphe graminis*) during growth in the glasshouse (Anon.1991). It was, however, subsequently included in Experiments 3, 4 and 5, along with Pastiche and Riband, with a prophylactic disease control programme used in the glasshouse during development.

Growing conditions for the plants are illustrated in Table 4.1. Tillers were periodically removed during development to restrict each plant to a main stem and three tillers. The plants were watered with tap water at ground level and received application(s) of fungicide (Corbel ®, a.i.= fenpropimorph) and aphicide (Aphox® , a.i.= pirimicarb) during development when disease/pest occurrence was noted. The main stem of each plant was tagged at anthesis (ZGS 61) and the plants were then transferred to the controlled-environment cabinets for experimentation.

4.2.2 Controlled-environment cabinets

Studies were undertaken in Conviron ® (S10H) (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) controlled-environment cabinets (Plate 4.1). The floor size of the cabinets (0.75 m x 1.23 m) limited the number of plants (maximum of 60) which could be accommodated in the experiments. This restricted the number of experimental

Table 4.1 : Growth conditions, up to anthesis, of plants used in controlled-environment cabinet experiments.

Stage	Germination	Vernalisation	Post-Vernalisation
Site	Glasshouse	Fridge	Glasshouse
Plant Stage	Seed ↓ ZGS 12	ZGS 12-14	ZGS 12-14 ↓ ZGS 61
Rooting Medium	John Innes No. 2 Compost	John Innes No. 2 Compost	John Innes No. 2 Compost
Container	20 x 36 cm Tray	20 x 36 cm Tray	10 cm diameter Pot
Depth of planting (cm)	3	3	3
Number of plants / container	500	500	1
Temperature °C (Maximum / Minimum)	25 / 5	6 / 3	25 / 5
Photoperiod- Light (hours)	12-16	8	12-16
Number of applications of Water / week	2	1	2-4
Duration (Days)	7-10	60-70	Until Anthesis

treatments and limited replication in the experiments. Additionally, limited availability of a number of cabinets for extended periods of time also restricted experimental designs to

Plate 4.1 : Conviron® controlled-environment cabinet.

the use of two environments. Reliability of cabinets was also problematical with data from one experiment completely lost due to a temperature fault in a cabinet and one experiment only partially successful due to temperature regulation problems.

4.2.2.1 Light

Lighting in the cabinets was provided by twelve 115 W cool white fluorescent tubes (VHO-Sylvannia) and nine incandescent 25 W light bulbs (Crompton, Australia). Light duration in cabinets was set at 16 hours with an abrupt change to an 8 hour dark period. The photosynthetic photon flux density (PPFD) in the cabinets was measured using a quantum sensor (Delta-T Devices, Burwell, Cambridge) at ear height, at the start

of each experiment. In each experiment a similar PPFD (±10 μmol m^{-2} s^{-1}) was obtained by varying the distance of the plants from the light source, by altering the height of the cabinet shelving. It was, however, not possible to ensure equal PPFD between experiments.

4.2.2.2 Temperature and humidity

Two different temperature regimes were used in the cabinets during the experiments. The choice of these temperature regimes was based on possible conditions which may be experienced by cultivars grown under UK weather conditions. A high temperature regime similar to temperature extremes experienced in UK field situations in fine, hot summers e.g. 1984, (Anon., 1984) and July 1995 (Table 3.12) was selected for Cabinet H. A lower temperature regime similar to cool conditions sometimes experienced in UK field situations e.g. 1985 (Anon., 1985), and July 1996 (Table 3.13) was selected for Cabinet C. In Cabinet H temperature was set, so that during the light period the temperature was 26 (±2) °C and during the dark period it was 20 (±2) °C. In Experiments 4 and 5 the higher temperature was decreased to 25 (±2) °C to try and alleviate temperature fault problems experienced in the Cabinet H. In Cabinet C the air temperature at the top of the ears was set at 16 (±2) °C during the light period and 10 (±2) °C during the dark period. Diurnal temperature range in both environments was therefore kept similar at 6 (±2) °C. Plants were watered twice daily with tap water at soil level to avoid water stress in Cabinet H. Relative humidity was maintained at 85% (±15%) in both cabinets to establish slow grain drying conditions thought to be conducive to high PMAA (Gale *et al.*,1983). The water reservoir in Cabinet H required daily filling to maintain the high humidity conditions in the higher temperature regime, with the reservoir in Cabinet C requiring filling every three days.

4.2.3 Experimental treatments applied

Plants were transferred between temperature regimes (Cabinet C→H→C or H→C→H) at specific stages in grain growth using a developmental timescale based on °C-days after anthesis (base temperature 0 °C). As only two controlled-environment cabinets were used in the experiments, it was not possible to replicate the environments in each experiment, so a randomised block design inside the cabinets was used as the basis for replication within experiments. Sixty plants arranged in 12 rows of five plants were used in Experiments 1 and 2, with two rows corresponding to a block in the cabinet (6 blocks). In Experiments 3 and 4 the design of the experiments was altered with plants arranged in eight rows of six plants, with two rows equating to a block (4 blocks), with space left in each cabinet for the reciprocal transfer of plants. In Experiment 5, the design again altered with plants arranged in 10 rows of five plants, with two rows equating to a block (5 blocks).

The size of cabinets limited the number of plants used in experiments, preventing detailed assessments of growth stage and moisture content of the grains during development. Assessment of ZGS at time of transfer between environments was made by subjective assessment of four grains per ear. An assessment of moisture content of the grains at time of transfer was obtained by freeze drying four grains per ear. The actual times of transfer of plants and cultivars used in each of the experiments undertaken is shown in Table 4.2.

In Experiment 1, transferring plants from 16 °C light /10 °C dark to 26 °C light / 20 °C dark temperature regime (C→H→C) for a 72 hour (3 day) period, at four stages in grain development , was investigated (Table 4.2) . Six plants of the cultivars Pastiche and Riband were transferred at each developmental stage with all the plants harvested

Table 4.2 : Treatments used in controlled-environment cabinet experiments.

Number Start [a] / End [b] date	Cultivar	Length of transfer time [c]	Treatment growth stage (°C-days after anthesis)	Treatment growth stage (ZGS)	Mean moisture content	Photosynthetic photon flux density (μmol m^{-2} s^{-1})
1	Pastiche	72 hours C→H→C	560	75-77	58	204
	Riband		630	77-83	49	
Feb 94			700	85-87	37	
-						
Aug 94			798	87	20	
2	Pastiche	72 hours C→H→C	324	73	72	200
	Riband		390	73-75	68	
Nov 94			488	75	59	
-						
May 95			739	83	37	
3	Hornet	72 hours C→H→C	414	73-75	63	450
	Pastiche		500 [d]			
			600 [d]			
Feb 95	Riband	72 hours H→C→H	405	73-75	64	
-			500 [d]			
Aug 95			600 [d]			
4	Hornet	72 hours C→H→C	518	73	64	350
	Pastiche		645	77	55	
	Riband		811	83	30	
		72 hours H→C→H	529	73	63	
			644	77	48	
Oct 95						
-			805	83	26	
May 96						
5	Hornet	120 hours C→H→C	608	77	51	250
	Riband		702	83	39	
		240 hours C→H→C				
		120 hours H→C→H	611	77	47	
			728	83	35	
Jan 96		240 hours H→C→H				
-						
Aug 96						

[a] Date when seed initially sown, before vernalisation, [b] Date of harvest of plants in controlled-environment cabinets, [c] H = 26 °C light / 20 °C dark, C = 16 °C light / 10 °C dark, [d] Cabinet fault prevented all treatments being applied.

after 980 °C-days (63 DAA). Table 4.3 illustrates the skeleton ANOVA table used in the analysis.

Table 4.3 : Skeleton analysis of variance table for controlled-environment cabinet Experiments 1 and 2.

Source of variation	df
Blocks	5
Treatment	1
Treatment / Time of transfer	3
Treatment / Cultivar	2
Treatment / Time of transfer.cultivar	3
Error	286
Total	299

In Experiment 2 the effect of transferring plants from 16 °C/10 °C to 26 °C/ 20 °C temperature regime (C→H→C) for a 72 hour period at earlier stages in grain development, was investigated (Table 4.2). Six plants of Pastiche and Riband were again transferred at each developmental stage with all the plants harvested after 1160 °C days (81 DAA). The same skeleton ANOVA table as used in the statistical analysis of Experiment 1 was used (Table 4.3).

In Experiment 3, three cultivars, Hornet, Pastiche and Riband were included in the experimentation, with four plants transferred reciprocally between cabinets (C→H→C and H→C→H) at each grain developmental stage (Table 4.2). Transfer at only one plant developmental stage was however successful due to a temperature fault in the 26 °C/ 20 °C temperature regime in Cabinet H. The plants were harvested after 1022° C-days (73 DAA) in Cabinet C and 1163 °C-days (53 DAA) in Cabinet H. Table 4.4 illustrates the skeleton ANOVA used in statistical analysis.

In Experiment 4 the reciprocal transfers (C→H→C and H→C→H) of three cultivars, Hornet, Pastiche and Riband at three different stages during grain development was undertaken, with four plants transferred at each stage in grain development (Table

4.2). The plants were harvested after 1106 °C-days (79 DAA) in Cabinet C and 1150 °C-days (50 DAA) in Cabinet H. Table 4.5 illustrates the skeleton ANOVA used in the statistical analysis.

Table 4.4 : Skeleton analysis of variance table for controlled-environment cabinet Experiment 3.

Source of variation	df
Blocks	3
Treatment	1
Treatment / Time of transfer	2
Treatment / Cultivar	4
Treatment / Time of transfer.Cultivar	4
Error	276
Total	287

Table 4.5 : Skeleton analysis of variance table for controlled-environment cabinet Experiment 4.

Source of variation	df
Block	3
Treatment	1
Treatment / Temperature	2
Treatment / Time of transfer	2
Treatment / Cultivar	4
Treatment / Temperature.Time of transfer	2
Treatment / Cultivar.Time of transfer	4
Error	73
Total	95

In Experiment 5 the time of transfer between the 16 °C / 10 °C and 26 °C / 20 °C temperature regimes (C→H→C and H→C→H) was increased to 120 hours (five days) and 240 hours (ten days), with transfers being undertaken at two stages in grain development (Table 4.2). Two cultivars, Hornet and Riband, were included in this experiment, with five plants transferred at each stage in grain development. Plants were harvested after 1150 °C-days (85 DAA) in Cabinet C and 1175° C-days (50 DAA) in Cabinet H. Table 4.6 shows the skeleton ANOVA table used in the statistical analysis of results.

Table 4.6 : Skeleton analysis of variance table for controlled-environment cabinet Experiment 5.

Source of variation	df
Block	4
Treatment	1
Treatment / Temperature	2
Treatment / Cultivar	2
Treatment / Time of transfer	1
Treatment / Length of transfer	1
Treatment / Temperature.Cultivar	2
Treatment / Temperature.Time of transfer	1
Treatment / Cultivar.Time of transfer	1
Treatment / Temperature.Length of transfer	1
Treatment / Cultivar.Length of transfer	1
Treatment / Time of transfer.Length of transfer	1
Error	81
Total	99

Grains were harvested in the experiments when the ears were yellow and grains were hard (ZGS 92), with the grains removed from the ears by hand. This subjective analysis of harvest ripeness led to slight differences in actual harvest date between experiments. Harvested grains were subsequently freeze-dried and stored in a freezer before analysis. The grain moisture content of harvested grains, measured by freeze drying, was 12 (±2) %.

4.2.4 *Alpha*-amylase activity assay

An air-segmented flow autoanalyser (Skalar (UK) Ltd ,York, UK.) was used to measure *alpha*-amylase activity as described in Section 3. 2.10. *Alpha*-amylase activity in single grains from florets 1 and 2, of the central three spikelets of the main ear was measured in Experiments 1,2 and 3. In Experiments 4 and 5, grains from the whole ear were used.

Assays were initially performed on single grains from defined spikelet positions to establish the amount of variability in *alpha*-amylase activity between grains of a similar

developmental age. This also allowed indirect examination of the hypothesis that grain size is linked to *alpha*-amylase activity with grain weight as opposed to grain size being compared to *alpha*-amylase activity (Evers *et al.*, 1995). A change from single grain assays, to assays of grain from the whole ear was made because it was felt using whole ears would reflect the situation experience by cultivars in the field more accurately and would be more informative in the development of a prediction model. Additionally, performing single grain assays was time consuming in nature and relatively expensive (primarily due to the cost of the *beta*-limit dextrin substrate).

Sample extracts from single grains were prepared by grinding the freeze dried single grains in a pestle and mortar. The grain was then carefully transferred to a 1.5 ml centrifuge tube and 1 ml of the extracting solution (5 g l^{-1} sodium chloride and 0.2 g l^{-1} calcium chloride) was added. The tubes were shaken on an orbital shaker (Gallenkamp, Leicester, UK) at 100 r min^{-1} for 5 minutes and then centrifuged at 2700 r min^{-1} for 5 minutes. The supernatant was then removed and assayed for *alpha*-amylase activity. Grain from whole ears was freeze-dried and then milled in a hammer mill (Falling Number AB, Stockholm, Sweden). Subsequently, 10 ml of extracting solution was then added to a 0.5 g flour sample and extracted as above, before the supernatant was assayed.

4.2.5 Sprouting assessment

Visual assessment of the grains was made to check for any signs of splitting of pericarp over the embryo indicating that sprouting had occurred (Wellington, 1956) . Additionally, five grains from each ear were examined for lipase activity as an indicator of sprouting using the fluorescent dye, fluorescein dibutyrate (Jensen *et. al.*,1984), as described in Section 3.2.12.

4.2.6 Iso-electric focusing

This was performed as described in Section 3.2.14, except *alpha*-amylase was extracted from a 0.15 g sample of freeze-dried grain which had been ground using a pestle and mortar.

4.2.7 Statistical analysis

Data was analysed using the statistical package Genstat, version 5 (Payne *et al.*, 1993). Specific *alpha*-amylase activities were transformed to a $\log_e$ scale to reduce the heterogeneity of variance before data analysis (Evers and Ferguson, 1980).

4.3 Results

4.3.1 *Alpha*-amylase activity

4.3.1.1 *Alpha*-amylase activity and grain weight

In Experiment 1 there was large variability in the freeze-dried weight of the harvested single grains, varying from 10.0 to 47.5 mg. Grains of Riband were significantly ($P = 0.006$) heavier than those of Pastiche. The *alpha*-amylase activity in each grain was also very variable, ranging from 0.15 to 255.2 mEU grain^{-1}. No relationship was identified between grain weight and *alpha*-amylase activity when all the data was pooled (Figure 4.1).

In Experiment 2 the grains were heavier than those in Experiment 1, with the freeze-dried weight of the grains varying from 20 to 72 mg, with grains of Riband again heavier than those of Pastiche ($P < 0.001$). The *alpha*-amylase activity in each grain ranged from 0.1 to 3607 mEU grain^{-1} with the *alpha*-amylase activity in grains of Riband higher than in those of Pastiche ($P < 0.001$). There was however no evidence of a relationship between grain weight and *alpha*-amylase activity within cultivars (Figure 4.2).

Figure 4.1 : Relationship between grain weight and *alpha*-amylase activity in the cultivars Pastiche and Riband in controlled-environment cabinet Experiment 1.

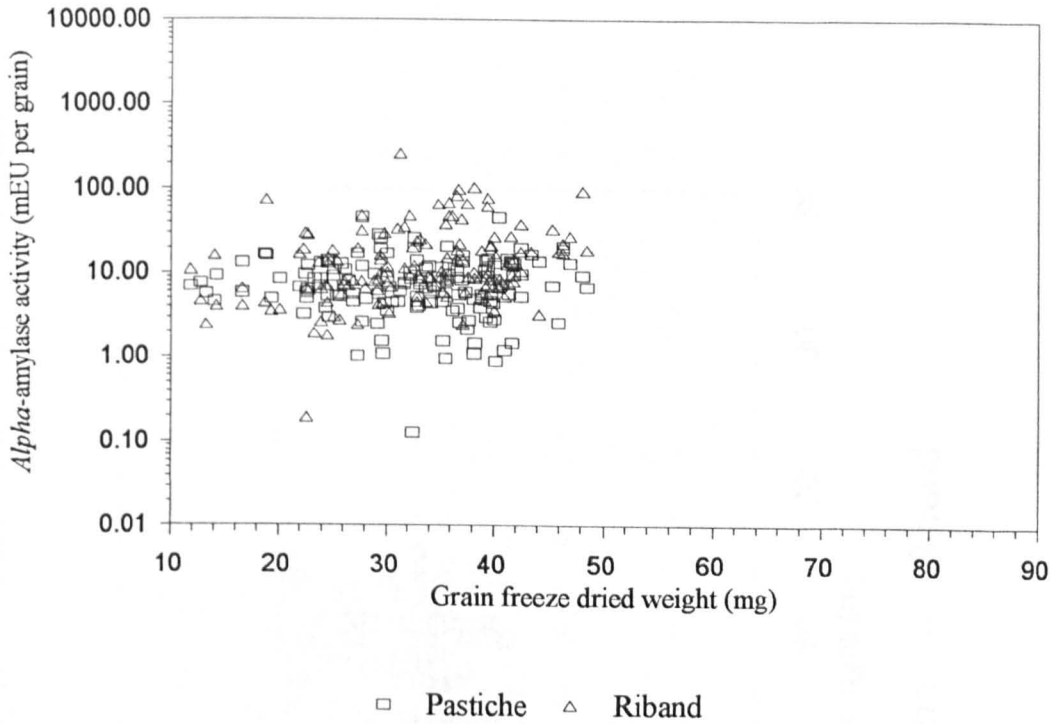


Figure 4.2 : Relationship between grain weight and *alpha*-amylase activity in the cultivars Pastiche and Riband in controlled-environment cabinet Experiment 2.

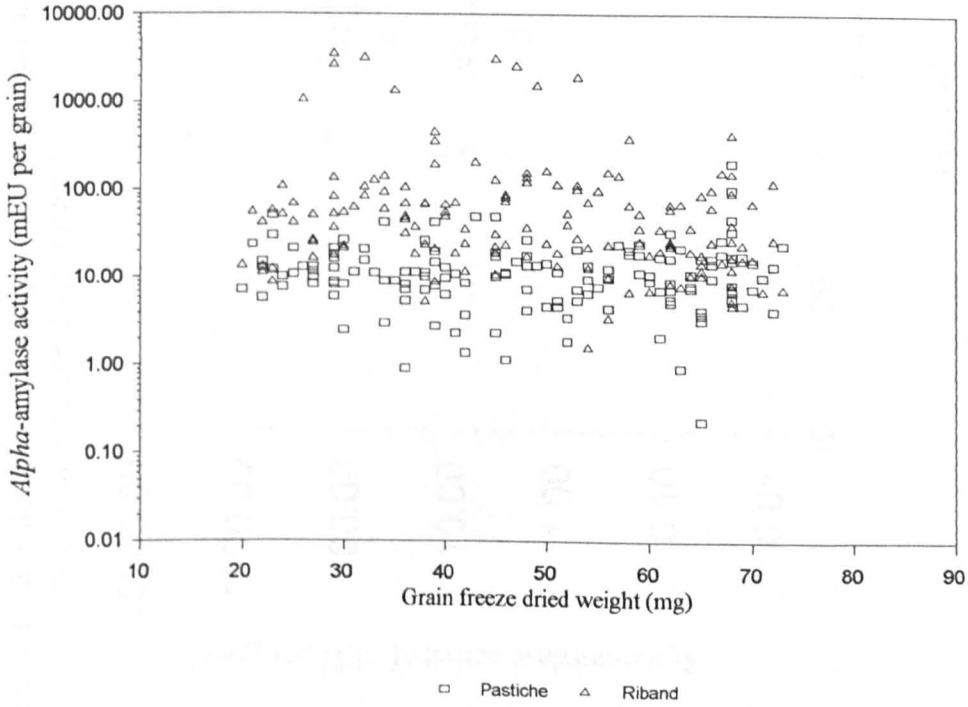
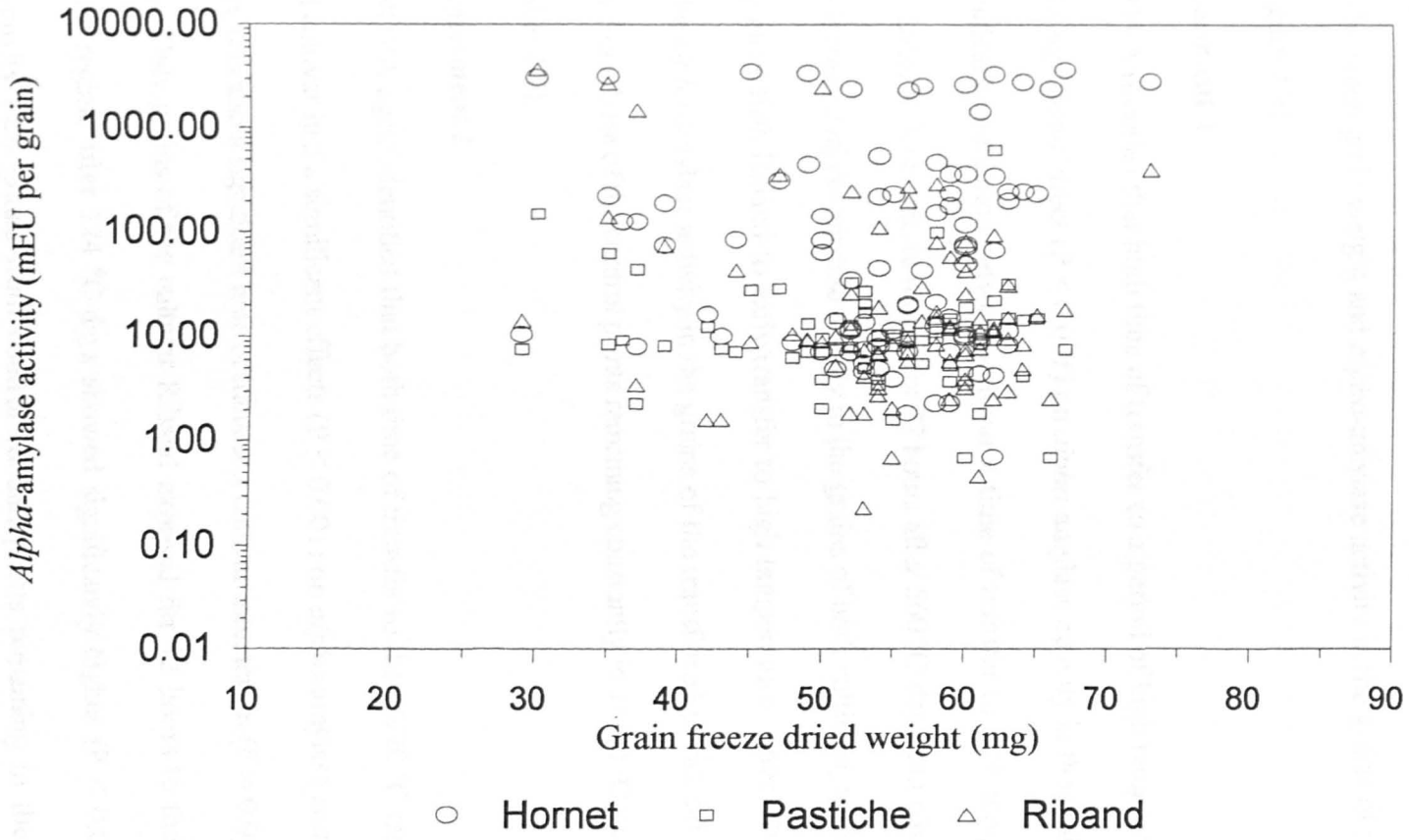


Figure 4.3 : Relationship between grain weight and *alpha*-amylase activity in the cultivars Hornet, Pastiche and Riband in controlled-environment cabinet Experiment 3.



In Experiment 3 the freeze-dried weight of the grains varied from 27 to 74 mg, with the *alpha*-amylase activity in each grain varying from 0.2 to 3652 mEU grain $^{-1}$. Hornet tended to have higher *alpha*-amylase activity than Riband, which in turn tended to have higher *alpha*-amylase activity than Pastiche. There was no evidence of a relationship between grain weight and *alpha*-amylase activity in the grains of the three cultivars (Figure 4.3).

4.3.1.2 Experiment 1

ANOVA revealed that both time of transfer to a period of high temperature and cultivar had a significant effect ($P < 0.001$) on *alpha*-amylase activity in the grain. There was no significant interaction between cultivar x time of transfer to high temperature. Transfers to 26/20 °C temperature regime for 72 hours after 560 °C-days and 630 °C-days significantly increased *alpha*-amylase activity in the grains of both cultivars, with Pastiche responding more than Riband to early transfer to high temperature. After 630 °C-days however, the *alpha*-amylase activity in the grains of the transferred plants did not differ significantly from those of the control plants remaining constantly in 16/10 °C temperature regime (Table 4.7).

4.3.1.3 Experiment 2

ANOVA again identified that both time of transfer to the 26/20 °C temperature regime and cultivar had a significant effects ($P < 0.001$) on *alpha*-amylase activity in the grain. There was also a significant time of transfer x cultivar interaction ($P = 0.047$) within treatments. Only grains of the cultivar Riband exposed for 72 hours to the 26/20 °C temperature regime after 324 °C-days showed significantly higher ($P < 0.05$) *alpha*-amylase activity than grains from control Riband plants remaining in the 16/10 °C temperature regime. No significant difference was seen in the *alpha*-amylase activity of

grains from control and treated Pastiche plants. Grains of Riband had significantly ($P < 0.05$) higher *alpha*-amylase activity than those of Pastiche, in both the grains of control plants kept solely at 16/10 °C, and in all the grains of plants exposed to a transient 72 hour period in the 26/20 °C temperature regime (Table 4.8).

4.3.1.4 Experiment 3

Exposure to a transient increase or decrease in temperature had no significant effects ($P = 0.136$) on *alpha*-amylase activity in the grains (Table 4.9). There was a significant ($P < 0.001$) cultivar effect, with grains of the cultivar Hornet having significantly ($P < 0.05$) higher *alpha*-amylase activity than those of Riband, which in turn had significantly ($P < 0.05$) higher *alpha*-amylase activity than those of Pastiche. This was apparent in both the grains of control plants kept solely in the 16/10 °C temperature regime, and in the grains of plants transferred for 72 hours to the 25/20 °C temperature regime. This is consistent with the results from Experiment 2 (Table 4.8).

There was no significant difference in the *alpha*-amylase activity between grains of Pastiche kept solely in 16/10 °C or 25/20 °C temperature regime. Grains from Riband and Hornet plants kept constantly in the 16/10 °C temperature regime did however have significantly higher ($P < 0.05$) *alpha*-amylase activity than their comparative plants kept constantly in the 25/20 °C temperature regime.

4.3.1.5 Experiment 4

ANOVA identified a significant difference ($P < 0.001$) in *alpha*-amylase activity between temperature treatments, with cultivar also having a significant effect ($P < 0.001$) on *alpha*-amylase activity (Table 4.10). There were also significant interactions between time of transfer x cultivar ($P < 0.014$), environment x cultivar ($P < 0.001$), and environment x cultivar x time of exposure to transient temperature change ($P < 0.001$).

Table 4.7 : The *alpha*-amylase activity in grains from plants of the cultivars Pastiche and Riband transferred from 16/10°C to 26/20°C controlled-environment cabinet for 72 hours at one of four stages during grain development in Experiment 1.

Temperature regime			16/10→26/20→16/10 °C C→H→C				16/10 °C C [a]	
Transfer time at	°C-days after anthesis		560	630	700	798		
	Days after anthesis		32	38	43	50		
	Moisture content (%)	Pastiche	58	48	36	22		
		Riband	58	50	38	18		
	ZGS		75-77	77-83	85-87	87		Overall mean
Harvest	*alpha*-amylase activity (mEU g^{-1} dry weight)	Pastiche	492	364	213	146	155	396
		Riband	482	438	347	260	334	594
		Mean	487	399	272	195	228	495
	alpha-amylase activity (log_e mEU g^{-1} dry weight)	Pastiche [b]	6.20	5.90	5.36	4.98	5.04	5.50 [d]
		Riband	6.18	5.99	5.92	5.56	5.81	5.90
		Mean [c]	6.19	5.94	5.64	5.27	5.43	5.69

[a] Control plants not transferred, [b] SED (between Time of transfer and Cultivar treatments) = 0.221, df = 285, [c] SED (between Time of transfer means) = 0.156, df = 285 , [d] SED (between Cultivar treatments) = 0.111, df=285, CV = 15.1 %.

Table 4.8 : The *alpha*-amylase activity in grains from plants of the cultivars Pastiche and Riband transferred from 16/10°C to 26/20°C controlled-environment cabinet for 72 hours at one of four stages during grain development in Experiment 2.

Temperature regime			16/10→26/20→16/10 °C C→H→C				16/10 °C C[a]	
Transfer time at	°C-days after anthesis		324	390	488	739	1160	
	Days after anthesis		25	30	37	52	81	
	Moisture content (%)	Pastiche	73	70	60	38		
		Riband	71	66	58	35		
	ZGS		73	73-75	75	83		Overall mean
Harvest	*alpha*-amylase activity (mEU g^{-1} dry weight)	Pastiche	346	277	263	196	328	282
		Riband	2591	1310	648	762	671	1196
		Mean	1469	794	456	479	500	739
	alpha-amylase activity ($\log_e$ mEU g^{-1} dry weight)	Pastiche [b]	5.85	5.62	5.57	5.28	5.79	5.62 [d]
		Riband	7.86	7.18	6.47	6.64	6.51	6.93
		Mean [c]	6.85	6.40	6.02	5.96	6.15	6.28

[a] Control plants not transferred, [b]SED (between Time of transfer and Cultivar treatments) = 0.638, df = 285, [c]SED (between Time of transfer means) = 0.452, df =285, [d] SED (between Cultivar means) = 0.319, df =285, CV = 20.0 %.

Table 4.9 : The *alpha*-amylase activity in grains from plants of the cultivars Pastiche, Riband and Hornet transferred reciprocally between a 16/10°C and a 25/20°C controlled-environment cabinet for 72 hours at one stage during grain development in Experiment 3.

Temperature regime			16/10→25/20→16/10 °C C→H→C	16/10 °C C [a]	25/20→16/10→25/20 °C H→C→H	25/20 °C H [a]
Transfer time at	°C-days after anthesis		414		405	
	Days after anthesis		30		19	
	Moisture content (%)	Pastiche	62		64	
		Riband	62		62	
		Hornet	64		67	
	ZGS		73		73	
Harvest	*alpha*-amylase activity (mEU g^{-1} dry weight)	Pastiche	237	150	156	237
		Riband	679	378	89	123
		Hornet	1808	2230	151	181
		Mean	908	919	132	180
	alpha-amylase activity (log_e mEU g^{-1} dry weight)	Pastiche	5.47	5.01	5.05	5.47
		Riband [b]	6.52	5.94	4.49	4.81
		Hornet	7.50	7.71	5.02	5.20
		Mean [c]	6.50	6.22	4.85	5.16

[a]Control plants not transferred, [b] SED (between Time of transfer and Cultivar means) = 0. 708, df =276, [c] SED (between Time of transfer means) = 0.254, df =276, CV= 21.9 %

Grains from control plants kept constantly in the 16/10 °C environment had higher *alpha*-amylase activity ($P < 0.05$) than grains kept constantly in the 25/20 °C environment. For plants kept constantly in the 25/20 °C environment, grains of the cultivar Hornet had higher ($P < 0.05$) *alpha*-amylase activity than those of Riband, which in turn had higher ($P < 0.05$) *alpha*-amylase than grains from Pastiche.

Averaged over cultivars, transient exposure of plants to a 16/10 °C temperature regime from 25/20 °C regime increased *alpha*-amylase activity ($P < 0.05$), with this effect greatest in grains of Pastiche treated at 805 °C-days. Similarly in grains of Pastiche, transient exposure to a 25/20 °C regime from a 16/10 °C regime at 518° C-days significantly increased ($P < 0.05$) *alpha*-amylase activity. Conversely in grains of Riband, transient exposure to 25/20 °C regime at 518 °C-days significantly decreased *alpha*-amylase activity. In Hornet, increases in *alpha*-amylase were seen at all transfer stages, in both environments, although these increases were not statistically significant.

4.3.1.6 Experiment 5

Cultivar had a significant effect on *alpha*-amylase activity in grains ($P < 0.001$) and there was a significant interaction between environment x cultivar ($P < 0.033$). Time of transfer and the length of transfer of plants (120 and 240 hours) had no significant effects on *alpha*-amylase activity. A period of 25/20 °C temperature significantly increased *alpha*-amylase activity in grain of the cultivar Hornet, compared to grains from plants kept solely in the 16/10 °C temperature regime. The reverse effect was seen in the cultivar Riband, with 25/20 °C temperature regime significantly decreasing ($P < 0.05$) *alpha*-amylase activity, compared to the grains from control plants kept solely at 16/10 °C. Transfers to five or ten day periods of 16/10 °C temperature did not significantly increase

Table 4.10 : The *alpha*-amylase activity in grains from plants of the cultivars Pastiche, Riband and Hornet transferred reciprocally between a 16/10°C and a 25/20°C controlled-environment cabinet for 72 hours at one of three stages in grain development in Experiment 4.

Temperature regime			16/10→25/20→16/10 °C C→H→C			16/10 °C C [a]		25/20→16/10→25/20 °C H→C→H			26/20 °C H [a]	
Transfer at	°C-days after anthesis		518	645	811			529	644	805		
	Days after anthesis		37	46	58			23	28	35		
	Moisture content (%)	Pastiche	65	57	33			61	52	28		
		Riband	64	55	29			64	48	26		
		Hornet	62	52	29			64	44	24		
	ZGS		73	77	83		Mean	73	77	83		Mean
Harvest	*alpha*-amylase activity (mEU g^{-1} dry weight)	Pastiche	38	13	11	10	18	12	15	106	11	36
		Riband	82	488	376	425	343	23	27	23	15	22
		Hornet	695	511	435	350	498	366	447	348	213	343
		Mean	264	329	266	286	286	115	145	141	134	134
	alpha-amylase activity ($\log_e$ mEU g^{-1} dry weight)	Pastiche [b]	3.45	2.49	2.31	2.29	[d]2.64	2.39	2.71	3.60	2.21	[d]2.73
		Riband	3.65	6.17	5.88	5.22	5.23	3.02	2.96	2.97	2.68	2.91
		Hornet	6.49	6.22	6.04	5.82	6.14	5.78	5.84	5.63	5.35	5.65
		Mean [c]	4.53	4.96	4.74	[e]4.45	4.67	3.73	3.84	4.07	[e]3.41	3.76

[a]Control plants not transferred, [b] SED (between Time of transfer and Cultivar means) = 0.466 , df = 69, [c] SED (between Time of transfer means) = 0.269, df = 69, [d] SED (between Cultivar means) = 0.507, df = 69, [e] SED (between base environment means) = 0.134, df = 69, CV = 17.0 %

Table 4.11 : The *alpha*-amylase activity in grains from plants of the cultivars Riband and Hornet transferred reciprocally between a 16/10°Cand a 25/20°C controlled-environment cabinet for either 120 or 240 hours at one of two stages during grain development in Experiment 5.

Temperature regime			16/10→25/20→16/10 °C C→H→C				16/10 °C [a]C	25/20→16/10→25/20 °C H→C→H				26/20 °C [a]H
Transfer at	°C-days after anthesis		608		702			611		728		
	Days after anthesis		45		52			26		31		
	Moisture content (%)	Riband	50		37			45		33		
		Hornet	52		40			48		36		
	ZGS		77		83			77		83		
	Length of transfer (hours)		120	240	120	240		120	240	120	240	
Harvest	*alpha*-amylase activity (mEU g^{-1} dry weight)	Riband	32	52	28	44	39	31	23	23	25	26
		Hornet	937	384	121	414	464	130	238	168	46	146
		Mean	484	218	75	229	251	80	131	95	35	86
	alpha-amylase activity (log_e mEU g^{-1} dry weight)	[b] Riband	2.72	2.99	2.83	3.45	[d] 3.00	3.29	3.08	2.84	3.07	[d] 3.07
		Hornet	5.77	5.41	4.60	4.59	5.09	4.65	4.89	4.94	3.76	4.56
		[c] Mean	4.25	4.20	3.71	4.02	[e] 4.05	3.97	3.98	3.89	3.41	[e] 3.82

[a]Control plants not transferred, [b] SED (between Time of transfer and Cultivar means) = 0.683, df = 72, [c] SED (between Time of transfer means) = 0.483, df = 72 , [d] SED(between Cultivar means) = 0.306, df = 72,[e] SED(between base environment means) = 0.216, df = 72, CV = 27.5 %

alpha-amylase activity in grains of either Hornet or Riband. There was no significant difference in *alpha*-amylase activity of grains from Hornet and Riband plants exposed to transient changes in temperature from 16/10 °C or 25/20 °C. Grains from Hornet plants exposed to temperature changes did, however, have higher *alpha*-amylase activity than Riband plants exposed to similar transfers. There was no significant difference between the *alpha*-amylase activity of grains from plants kept solely in the 16/10 °C and 26/20 °C temperature regimes, except that *alpha*-amylase activity in Riband plants kept solely in the 26/20 °C temperature regime, was significantly lower ($P < 0.05$) than the other controls (Table 4.11).

4.3.1.7 Variability between experiments

The coefficient of variation in Experiments 1, 2 and 3, where *alpha*-amylase activity assays were undertaken on single grains, varied from 15.1 to 21.6 %. In Experiments 4 and 5, where *alpha*-amylase activity was measured in grain from the whole ear, the coefficient of variation ranged from 17.0 to 26.6% (Table 4.12).

The variation in activity of control plants has made comparisons between experiments problematical. To allow clearer visualization of the results, *alpha*-amylase activity has been expressed as a percentage of the *alpha*-amylase activity in its respective control treatments in each experiment.Figure 4.5 illustrates the combined results from Experiments 1, 2, 3 and 4, showing the effect of a transient period of exposure to high temperature on *alpha*-amylase activity. It is evident that larger and more variable changes in PMAA occur in response to transient increases in temperature change at grain moisture contents greater than 60%, with the magnitude of the responses decreasing as grain moisture content declines to 40% and below. Figure 4.6 illustrates the combined results from Experiments 3 and 4, showing the effect of a transient period of exposure to low

Figure 4.5 : Pooled data from controlled-environment cabinet Experiments 1, 2, 3 and 4, showing the effect of a transient 72 hour increase in temperature from a 16 / 10 °C to a 25 or 26 /20 °C temperature regime, at different stages during grain development, on *alpha*-amylase activity in the grain at harvest.

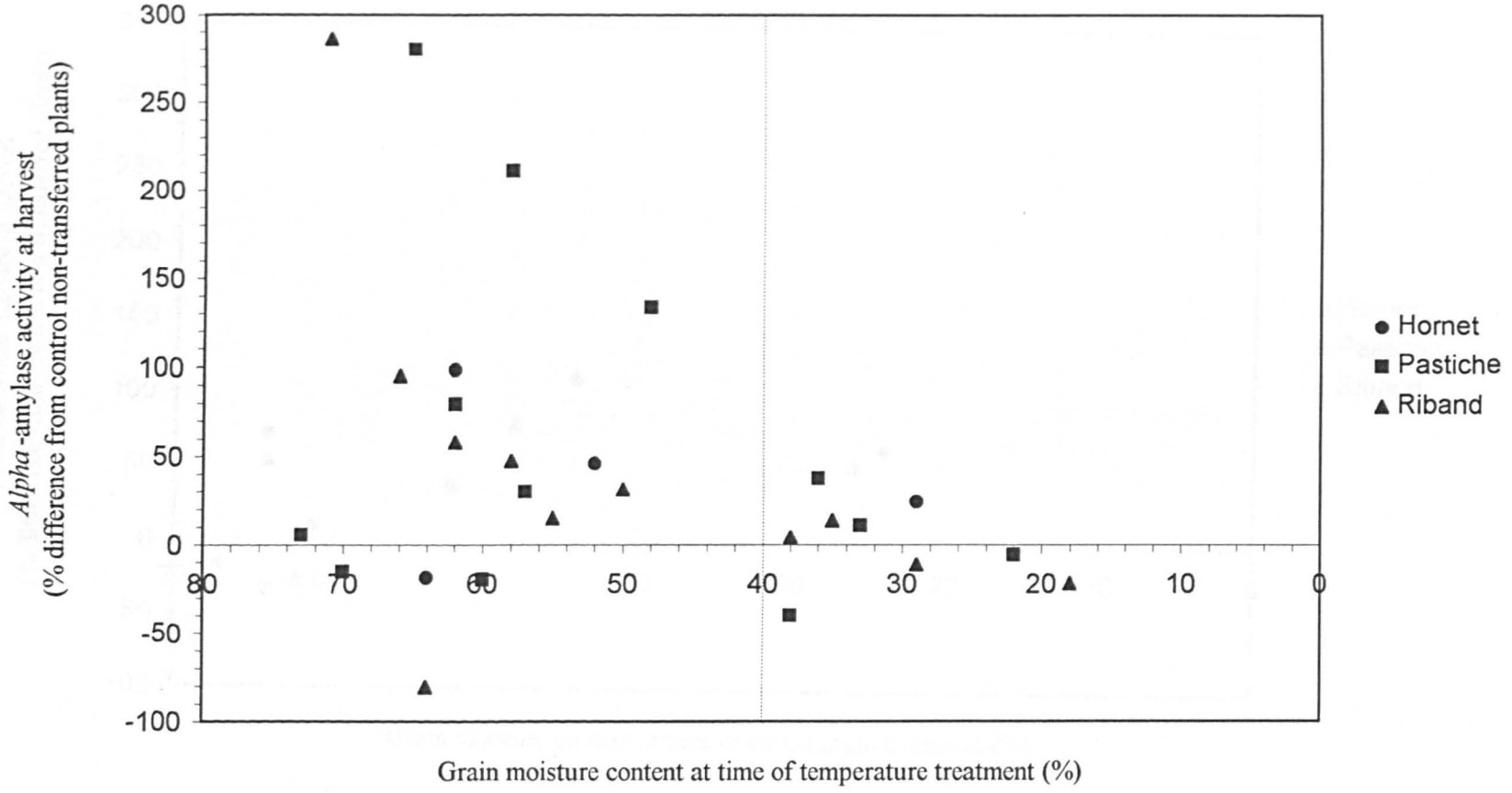


Figure 4.6 : Pooled data from controlled-environment cabinet Experiments 3 and 4, showing the effect of a transient 72 hour decrease in temperature from a 25 / 20 °C to a 16 /10 °C temperature regime, at different stages during grain development, on *alpha*-amylase activity in the grain at harvest.

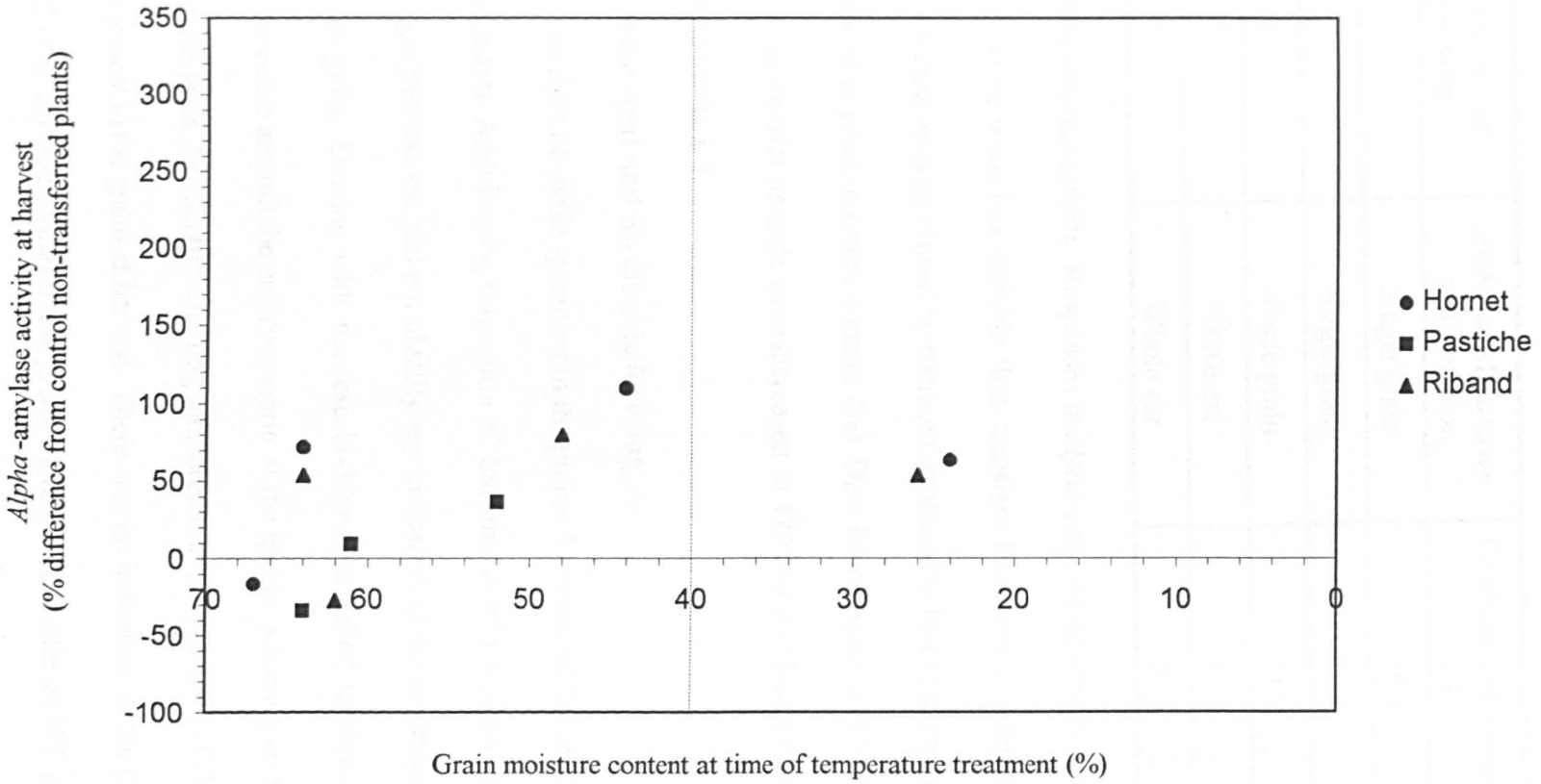


Table 4.12 : Variability between controlled-environment cabinet experiments.

Controlled-environment cabinet experiment	*Alpha*-amylase assay performed on	Coefficient of variation (%)
1	Single grain	15.1
2	Single grain	20.0
3	Single grain	21.9
4	Whole ear	17.5
5	Whole ear	27.5

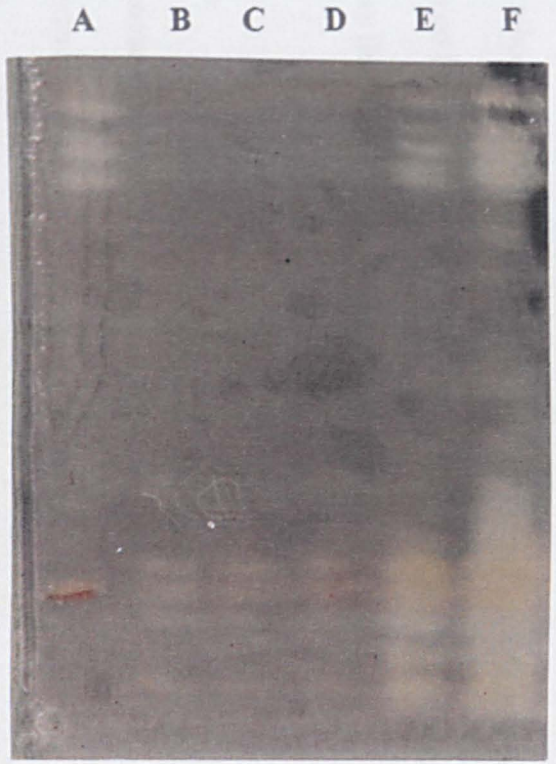
temperature on *alpha*-amylase activity. Responses in *alpha*-amylase activity to transient decreases in temperature were less variable than transient increases in temperature. Increases in *alpha*-amylase activity caused by transient exposure to low temperature did not appear to be related to grain moisture content (%). Data from Experiment 5 was not included in Figures, as transfer periods were increased to 120 and 240 hours compared to 72 hours in Experiments 1-4.

4.3.2 Sprouting assessment and iso-electric focusing

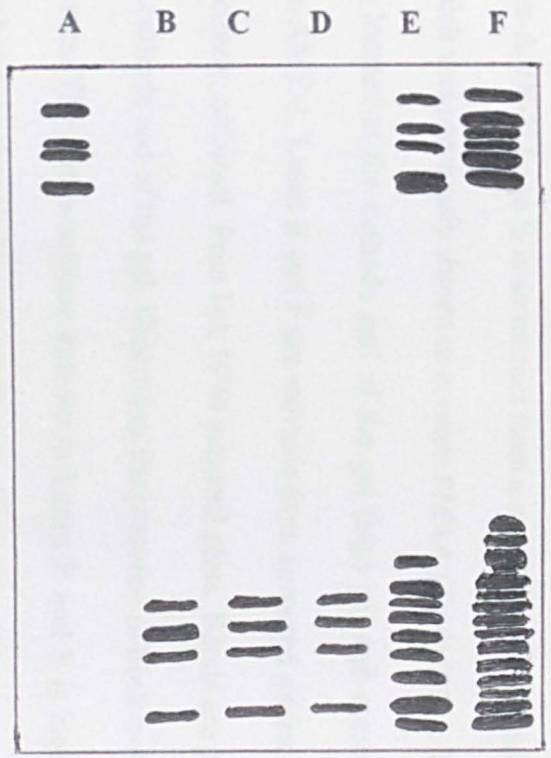
There were no signs of visible sprouting in the grains, from any of the controlled-environment experiments. Additionally, inspection of the embryo of a random selection of grains, using a light microscope, did not identify any indication of the commencement of sprouting in the grain. Staining with fluorescein-dibutyrate failed to identify any concentrated fluorescence around the embryo region of the grains indicating an absence of lipase activity in the grain. Iso-electric focusing indicated that only α-AMY-1 (high pI) isoenzymes were present in the grains at harvest. There was no indication of the presence of any α-AMY-2 (low pI) isoenzymes in the grain. Plate 4.2 illustrates an IEF gel from Experiment 1. Lane A on the gel is an extract from immature green grains of Riband-HA-1994 illustrating 4 bands at the anode end of the gel only (low pI) corresponding to four

Plate 4.2 : Iso-electric focusing gel from controlled-environment Experiment 1 illustrating the presence of α-AMY-1 isoenzymes.

Actual IEF gel photo



Schematic representation



Key
A : Control : Riband HA 1994, green immature grain (ZGS 77) (RPAA)
B : Control : Hornet HA 1994, combine sample of unsprouted grain (PMAA)
C : Experiment 1 : Riband transferred at a 560 °C-days after anthesis from C→H→C
D : Experiment 1 : Pastiche transferred at a 560 °C-days after anthesis from C→H→C
E : Control : Riband HA 1994, sprouted grain from irrigated plots (PoMS)
F : Control : Pastiche HA 1994, sprouted grain from irrigated plots (PoMS)

isozymes of α-AMY-2. Lane B is an extract from a combine harvest sample of Hornet HA 1994, which was previously shown to contain PMAA (Table 3.23). The four bands in lane B are located at the cathode end of the gel (high pI) and correspond to four isozymes of α-AMY-1. Lanes E and F are extracts from sprouted grains of Riband and Pastiche respectively, obtained from HA 1994 irrigated plots. Bands are present at both the anode and cathode end of the gel, illustrating the presence of both α-AMY-1 and α-AMY-2 isozymes. The *alpha*-amylase activity in Lanes E and F is high leading to a smearing of the bands on the gel. This makes the number of isozymes present at high and low pI difficult to distinguish in samples with PoMS. Lane A is acting as a control for RPAA, Lane B is acting as a control for PMAA and Lanes E and F are acting as controls for PoMS. Lane C contains an extract from grains of Riband from in Experiment 1, transferred between 16 / 10 °C and 26/ 20 °C environments for 72 hours at 560 °C days after anthesis, and Lane D contains a similar extract from Pastiche. Four faint bands are visible at the cathode end of the gel (high pI) in both lanes C and D, in a pattern similar to Lane B, with no bands seen at the anode end of the gel (low pI). The pattern in Lanes C and D mirrors that seen in Lane A suggesting that PMAA is present.

4.4 Discussion

4.4.1 *Alpha*-amylase activity and grain weight

The relatively low freeze-dried weight of the harvested grain from Experiments 1 and 2 can be attributed to the relatively low (204-200 μmol m^{-2} s^{-1}) PPFD in the cabinets which would limit photosynthesis. This is a common problem encountered in many controlled-environment cabinets (Downs and Hellmers, 1975). Using new replacement lights in Experiment 3 gave a higher PPFD (450 μmol m^{-2} s^{-1}) in the cabinets

and probably accounted for the higher proportion of heavier grains. The large variability in the *alpha*-amylase activity in the grains was surprising considering the grains were selected at the same developmental stage and from the same position in the ear This variability was, however, not as large as the variability found in grains from bulk samples (McVittie and Draper, 1982b).

Although grain weight and grain size are not directly comparable, heavier grains are normally associated with larger grains (Tashiro and Wardlaw, 1990). The large grained cultivar Hornet, had higher *alpha*-amylase activity than Riband or Pastiche reflecting NIAB score ratings (Anon.,1991). This supports the hypothesis that a relationship exists between genotype grain size and *alpha*-amylase activity, with larger grains having higher PMAA (Evers *et al.*, 1995). Recent field experiments, including the cultivar Hornet, have also shown that an increase in *alpha*-amylase activity per gram occurred as grain size increased (Naylor, 1999). The controlled-environment cabinet results do not, however, provide any support for the hypothesis that high *alpha*-amylase activity is linked to large grain size within genotypes. Additionally, short periods of high temperature tend to decrease grain size (Bhullar and Jenner, 1986) and would thus be expected to decrease *alpha*-amylase activity in the grain. In fact, increases in *alpha*-amylase activity occurred, suggesting a certain disparity with Evers *et al.* (1995). Further studies on grain size by Alberti *et al.* (1996) suggested increased grain size enhances the potential for high *alpha*-amylase activity in grains, but that this potential is not always realised in practice. This suggests other factors are having a greater effect on the expression of PMAA, with grain size being an additional influencing factor modulating PMAA which concurs with findings from the controlled-environment cabinet experiments.

4.4.2 Variability between experiments

The variability between experiments was relatively high, but not unsurprising considering the variability often seen in *alpha*-amylase activity in grain (McVittie and Draper, 1982b). Variations in the *alpha*-amylase activity of the control plants, which remained in the same temperature regime, has made comparisons between experiments awkward. To overcome this difficulty, results have been expressed as percentage of control activity in each experiment. This problem has also been seen in other studies where plants grown under identical conditions, from different crops sometimes produce different amounts of *alpha*-amylase following identical treatments (Cornford *et al.*, 1986). The conditions the plants were exposed to pre-heading may offer an explanation for this effect. Plants were grown in the glasshouse until anthesis and then transferred to controlled-environment cabinets for experimentation. Experiments were undertaken throughout the year, therefore the possibility that variations in light intensity and temperature in the glasshouse pre-anthesis may be affecting the plants can not be dismissed. Grain filling is affected by high temperatures (Wardlaw and Moncur, 1995) with the magnitude of response to high temperature modified by conditions during ear development. Low light intensity and high temperature prior to ear emergence have been shown to increase the tolerance of grains to post-anthesis high temperature stress (Wardlaw, 1994). This effect was postulated to be due to changes in the source-sink balance in the grain. Whether these effects also extend to affecting the grains sensitivity to produce PMAA is unknown.

4.4.3 Source of *alpha*-amylase in controlled-environment cabinet experiments

The high *alpha*-amylase activity detected in the grains was not due to germination, as no visibly sprouted grains in samples were seen. Additionally, staining

with fluorescein-dibutyrate did not indicate the presence of any lipase activity, indicative of PoMS (Jensen and Heltved, 1982). The high *alpha*-amylase activity in the grains cannot therefore be attributed to the high temperatures during grain development decreasing the length of dormancy (Belderok, 1968), leading to the sprouting of the grain in the ear, due to the high humidity conditions in the controlled-environment cabinets (King, 1993). The absence of α-AMY-2 (low pI) isoenzymes in the grain also suggests that RPAA was not the cause of the high *alpha*-amylase activity within the grain (Lunn *et al.*, 1997). The presence of α-AMY-1 isoenzymes in the grain and the absence of other indicators of PoMS or PrMS suggests that the high *alpha*-amylase activity in the grains can be assigned solely to PMAA.

4.4.4 The effect of a transient period of high temperature on PMAA

Overall, out of the 36 cultivar x time of transfer combinations undertaken from a 16/10 °C temperature regime to a 26/20 °C temperature regime in Experiments 1-5, six led to a significant ($P < 0.05$) increase in PMAA, one led to a decrease and 21 had no significant effect on PMAA. This compares to one significant increase in 20 which may be expected to occur by chance ($P < 0.05$). These results therefore provide some evidence that periods of high temperatures before the grain reaches physiological maturity can stimulate PMAA. This effect, however, does not appear to be consistent suggesting other factors are also playing a role in determining PMAA, illustrating that a complex interaction is occurring between the plant genotype and environmental temperature. The results support the hypothesis of Gold and Duffus (1996), that an early environmental event before physiological maturity is reached may stimulate high PMAA. In contrast, high June temperatures in the UK have been shown to be positively associated ($R^2 = 0.77$)

with high harvest HFN (low *alpha*-amylase activity) of UK wheat crops (Smith and Gooding, 1996). There therefore appears to be some disparity between the results, as the early plant developmental stages, shown to be sensitive to stimulation of high PMAA, by high temperature in the controlled environment experiments, would generally be expected to occur in the field, in late June or early July. The fact that Smith and Gooding (1996) did not take into account the actual grain developmental stage in their data analysis may explain some of the apparent differences between results. Additionally the difference may partly be explained by the high humidity conditions used in the controlled-environment cabinet experiments to ensure slow grain drying-rates which have been suggested to be optimal for PMAA (Gale *et al.*, 1983). In the absence of these conditions in the field, the effect of factors stimulating high PMAA may be concealed by a rapid grain drying-rate as has been suggested in previous experiments (Gold, 1991). This may be what commonly occurs under UK field conditions, with the problem of high PMAA only becoming apparent in certain years such as in 1985, when high temperatures early in grain development and slow grain-drying, led to the UK wheat crop only reaching an average HFN of 161, despite a low incidence of PoMS (Hough, 1990).

A possible role of high temperature in stimulating high PMAA in the grain is now tentatively proposed. Studies involving the crossing of the PMAA susceptible Australian cultivars Spica and Lerma 52 with near-isogenic lines carrying the GA-insensitive genes (*Rht 1, Rht 2 and Rht 3*) have confirmed that GA is involved in the expression of PMAA (Mrva and Mares, 1996a). Enhanced sensitivity of the aleurone layer to GA may thus cause higher PMAA. Sensitivity to GA which promotes *alpha*-amylase synthesis can be achieved in normally insensitive immature aleurone tissue of wheat grains by a high temperature (27 °C) treatment (Norman *et. al.*,1982). This effect is not however

consistent in inducing a GA-responsive state and does not fully reflect the response produced by the aleurone layer of a mature grain. It is thought this high treatment may be causing cells to respond to their own endogenous GA (Cornford *et al.*, 1986). High temperature may also cause premature drying of the developing aleurone layer. Premature drying has been shown to increase the GA-responsiveness of developing aleurone layers of barley (*Hordeum vulgare*) grain (Jiang *et al.*, 1996) and up-regulate *alpha*-amylase gene expression. Additionally it has been shown that exposure of wheat ears of the cv. Kolibri to high temperatures (20 °C and 25 °C), led to significantly higher amounts of GA in the grain 17 and 24 days after anthesis, compared to ears treated at 15 °C (Radley, 1976). Recent studies on *pga* (premature grain *alpha*-amylase) grain development mutants of barley, in which large amounts of PMAA are synthesised, revealed higher amounts of bioactive GA_1, compared to the "inactive" 2-*beta* hydroxylated GAs in the grain. A reduction in the extent of 2-*beta* hydroxylation was thus proposed as the cause of the increased level of GA_1 in the *pga* mutants, leading to high PMAA in these grains (Green *et al.*, 1997). By analogy with barley, high temperature may thus be affecting both the amount of GA in the developing wheat grain and the GA responsiveness of the aleurone layer. The soft-dough stage in grain development (ZGS 83) where the grain dry weight increases as the endosperm reserves accumulate more rapidly, appears particularly susceptible to stimulation of high PMAA. At this stage in grain development the aleurone layer is undergoing differentiation, with the embryo undergoing further differentiation (Briarty *et al.*, 1979; Simmonds and O'Brien, 1981). Possible changes in the fluidity of the aleurone cell membrane may be the crucial factor governing the expression of PMAA (Norman *et al.*, 1982; Hilhorst, 1998). A relationship between high abundance of lipid-transfer protein *(Ltp1)* mRNA, a protein in barley aleurone layer believed to be involved

in the transport of phospholipids in the lumen of the endomembrane system, and low dormancy has been postulated (Schuurink, 1993). The expression of *Ltp1* mRNA has been shown to be regulated by thermoperiodic conditions during grain maturation. The amount of LTP1 protein also correlated with the initial rate of GA-induced *alpha*-amylase secretion from isolated aleurone layers, suggesting LTP1 may share the same secretory pathway as *alpha*-amylase. This implies that high temperature may be causing an increased proliferation of GA_3 inducible secretion apparatus in the aleurone layer, or similar membrane proteins (Jones and Jacobsen, 1991). Whether this is also the cause of the generation of high PMAA in wheat is unknown. Additionally, high temperatures in grain development have been shown to inhibit the activity of the enzyme starch synthase, leading to an accumulation of sucrose in the grain endosperm (McLeod and Duffus, 1988). Sugars have been proposed as playing an important role in the germination of cereal grains. A tissue-specific interactive loop among sugars, gibberellins and *alpha*-amylase genes has been proposed as a control mechanism governing germination in rice (*Oryza sativa*) seeds (Yu *et al.*, 1996). Similarly sugar and hormonal regulation have been proposed to interact in regulation of GA-induced gene expression in barley, with a feedback control mechanism postulated to operate in the embryo, and an osmotic control mechanism thought to function in the aleurone cells (Perata *et al.*, 1997). These regulatory mechanisms may also play an important role early in wheat grain development, thus affecting the expression of PMAA.

The identification of high temperature as a stimulus for high PMAA under certain conditions has the potential to form the basis of an effective screening tool for distinguishing susceptibility to high PMAA in cultivars. However, the length of time of exposure to transient temperature change and the actual temperature range over which

the stimulatory effect on PMAA occurs needs to be more precisely defined before this test could be utilised.

4.4.5 The effect of a transient period of cool temperature on PMAA

Overall, out of the 18 cultivar x time of transfer combinations undertaken from a 25/20 °C temperature regime to a 16/10 °C temperature regime in Experiments 3-5, one led to a significant ($P < 0.05$) increase in PMAA, one led to a decrease and 16 had no significant effect on PMAA. This provides little evidence to support the hypothesis that a transient period of cool temperature before physiological maturity can stimulate PMAA (Mrva and Mares, 1994). as one increase in 20 would be expected to occur by chance at the 95 % probability level .Transferring the Australian cultivar BD-159 between a "high" and "low" temperature environment for 7 days at one of five developmental stages (7,14,21,28 and 35 DAA) identified significantly increased *alpha*-amylase activity in the grains of plants transferred after 21 and 28 DAA, when harvested at 42 DAA. Similarly in the cultivar Lancer, cool/wet conditions (20 °C/ 10 °C, 80-100 % relative humidity) during early stages of grain ripening, have been shown to lead to high α-AMY-1 activity in the grain (Nakatsu, 1999). The lack of a significant response in PMAA to a period of cool temperature in grain development before physiological maturity in the controlled environment-cabinet experiments is thus surprising, but may simply be due to different cultivars being used in the experiments.

4.4.6 The effect of duration of transient temperature change on PMAA

A 120 or 240 hour period of high or low temperature, at 608 or 611 °C days respectively, increased PMAA in Experiment 5, although this was not statistically significant compared to Experiments 3 and 4, where significant increases in PMAA activity were seen. The reason for this difference is not readily apparent although it

appears changing the length of exposure time from 72 hours in Experiments 3 and 4, to 120 and 240 hours in Experiment 5 may be an important factor governing expression of PMAA. It was anticipated that longer exposure to a transient 16/10 °C temperature regime would lead to increased PMAA, particularly as long periods of cool weather in the field have been associated with high PMAA (Mares and Gale, 1990), but this did not occur. A fall in PMAA due to the effect of a prolonged change to transient high temperature for 240 hours, is more easily reconciled. The longer period of high temperature is probably affecting the water relations in the grain and speeding up the grain drying-rate, thus counteracting any of the stimulatory effects of high temperature on PMAA before physiological maturity (Gold, 1991). Varying the length of exposure time at 700 °C-days had no significant effects on PMAA compared to controls, suggesting that this stage in grain development is insensitive to the effect of temperature fluctuations on PMAA.

4.5 Conclusion

To conclude, this series of experiments has demonstrated that PMAA can be increased by a transient increase in temperature before the grain reaches physiological maturity when coupled with subsequent slow grain drying conditions between 40-20 % grain moisture content. The experiments have also confirmed that variations in PMAA are not just attributable to variations in grain drying-rate, with a complex interaction between genotype and environmental temperature being one of the factors determining the amount of PMAA in grain.

5. The Development of a Model for Predicting High PMAA in UK Field Environmental Conditions.

5.1 Introduction

The results from the controlled-environment cabinet experiments (Section 4) supported the hypothesis that high PMAA can be stimulated by a period of high temperature at early stages in grain development, before the grain reaches a grain moisture content of 40% (approximately 700 °C-days after anthesis). This effect was, however, only thought to become apparent and have significant effects when coupled with slow grain drying conditions between 40-20% grain moisture content, i.e. in cool, high humidity conditions during the dough stage of grain development.

The aim of the analysis described in this section was to show whether the occurrence or absence of PMAA identified in the field experimental samples (Section 3) could be rationalised with the results obtained from the controlled environment-cabinet experiments (Section 4) and aid in the development of a meteorologically-based prediction scheme for the occurrence of high PMAA. This would be beneficial as it would largely remove the costly and labour-intensive stages of pre-harvest HFN sampling and subsequent sample analysis currently required for HFN prediction (Section 3). It would also probably enable predictions to be given further in advance, allowing growers more time to plan the harvesting, drying, storage and possible marketing of their grain (Kettlewell, 1993). Attempts at developing meteorologically-based HFN prediction schemes have been discussed in Section 2.10. None of the prediction schemes, however, considered the development of PMAA, with most focusing solely on the relationship between temperature and dormancy, and the role of PoMS in reducing HFN. The results from the field experiments (Section 3) confirmed that PMAA is an important factor

causing low HFN in UK winter wheat crops. Its inclusion in a meteorologically-based model predicting *alpha*-amylase activity and HFN is thus of importance if an accurate HFN prediction scheme is to be devised for UK field conditions.

5.2 Materials and methods

Weather data collected in the field experiments was used in the analysis (Section 3.2.6). The plant sampling timescale of °C-days after ear emergence used in the field experiments was converted to °C-days after anthesis, to allow easier comparison with data from the controlled-environment cabinet experiments. It was felt that °C-days after anthesis was a more appropriate developmental timescale to use in this comparison as it has been used more frequently by other experimental workers (Tottman *et al.*, 1985) than °C-days after ear emergence (Gate, P., personal communication).

Data was examined graphically to identify any putative relationships between environmental factors and the stages in plant development which had been shown to be susceptible to high PMAA in the controlled environment-cabinet experiments. The grain moisture content (%), maximum and mean temperature (°C) and relative humidity (%) were plotted against thermal time (°C-days after anthesis, base temperature 0 °C). A maximum temperature greater than 25 °C was assumed to be required to stimulate PMAA. This was derived from the transfers undertaken in the controlled-environment cabinet experiments (Section 4) where grains from plants transferred temporarily between 16/10 and 25/20°C temperature regimes, were shown to produce high PMAA. Relative humidity greater than 85%, as used in the controlled-environment cabinet experiments was also assumed to be required for high PMAA to become apparent in the field. Using these criteria, allowed a retrospective assessment of the likely occurrence or absence of

PMAA to be assigned. The occurrence or absence of these specified conditions in the field experiments was indicated respectively by a tick or cross in Tables 5.1-5.4, with the greater number of ticks, hypothesised to indicate a higher probability of high PMAA occurring. Grain drying-rate data taken from Table 3.15 was also considered in the assessments.

5.3 Results

Similar developmental rates in the four UK cultivars at each site (Table 3.14) meant that there was little difference in the environmental conditions experienced at set grain developmental stages. However, the weather experienced at different sites gave more variation in conditions at set grain developmental stages, allowing a better comparison of results. For this reason, attention is focussed primarily on Hornet, the cultivar most susceptible to PMAA, with data concerning Riband, Haven and Pastiche presented in less detail.

5.3.1 Occurrence of PMAA in Hornet

Figures 5.1-5.3 illustrate the maximum and mean temperature, the relative humidity and moisture content changes during grain development of the cultivar Hornet at HA 1994, AB 1995 and AB 1996 respectively, where PMAA was detected. The absence of relative humidity data from UA 1996 prevented the display of data from this site, where PMAA also occurred.

At HA 1994, maximum temperatures greater than 25 °C, occurred around 500 and 600 °C-days after anthesis, with periods of high humidity also seen after 500 and 600 °C-days, (Figure 5.1) . These conditions were coupled with a slow grain drying-rate of 1.86% moisture loss day^{-1}. All conditions for the hypothesised occurrence of PMAA were

Figure 5.1 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at HA in 1994, where PMAA was detected.

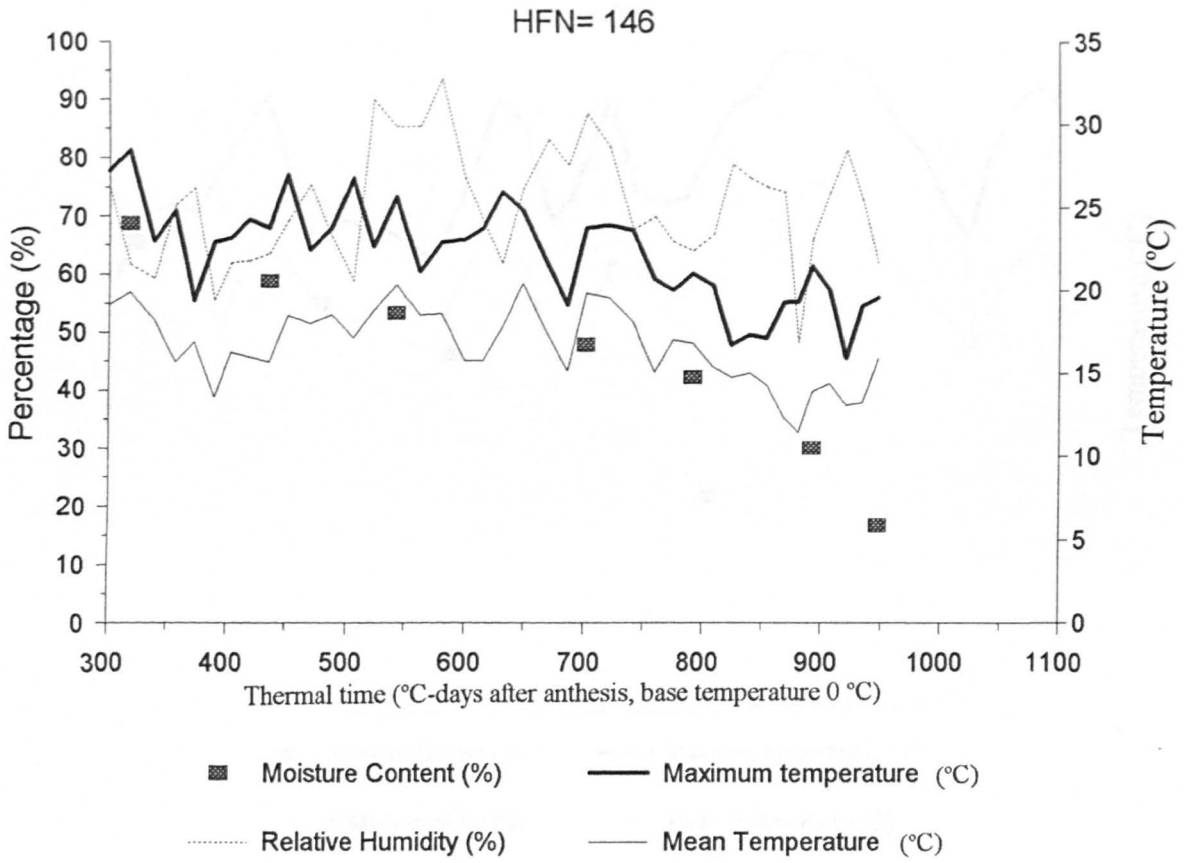


Figure 5.2 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at AB in 1995, where PMAA was detected.

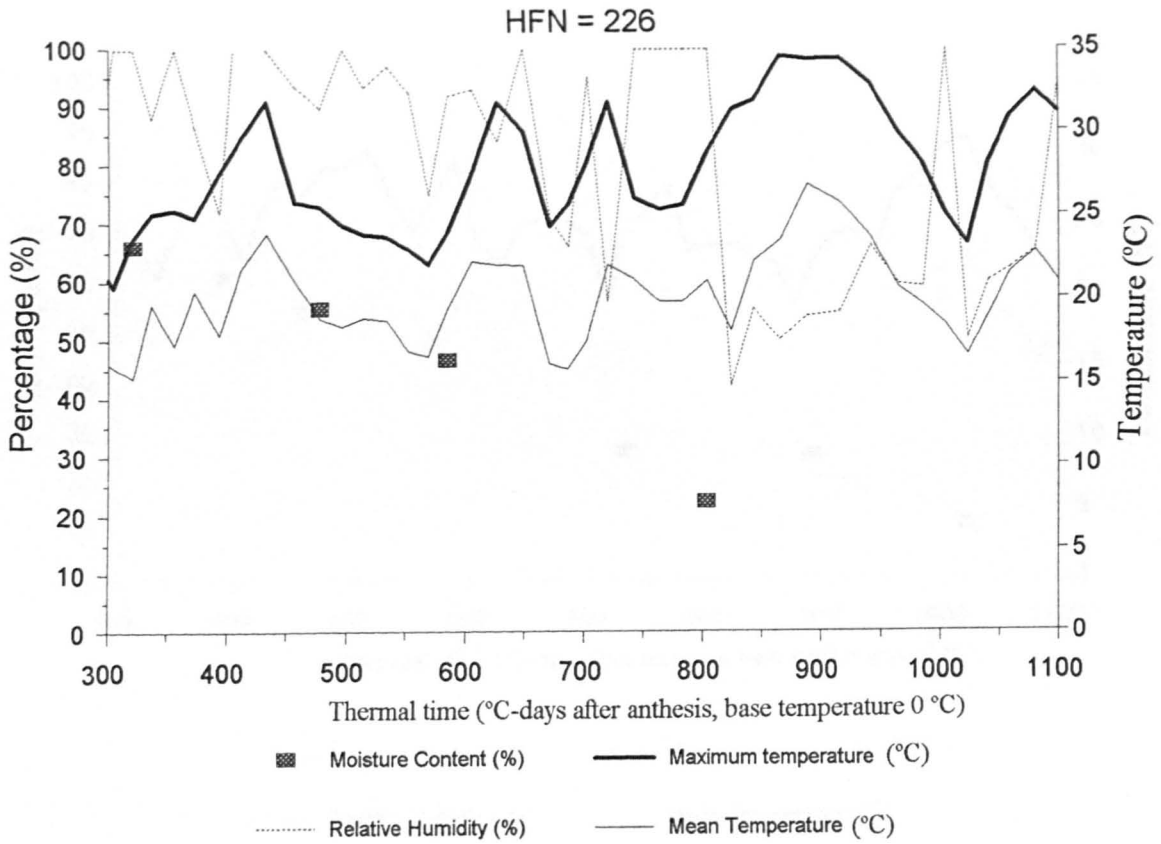


Figure 5.3 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at AB in 1996, where PMAA was detected.

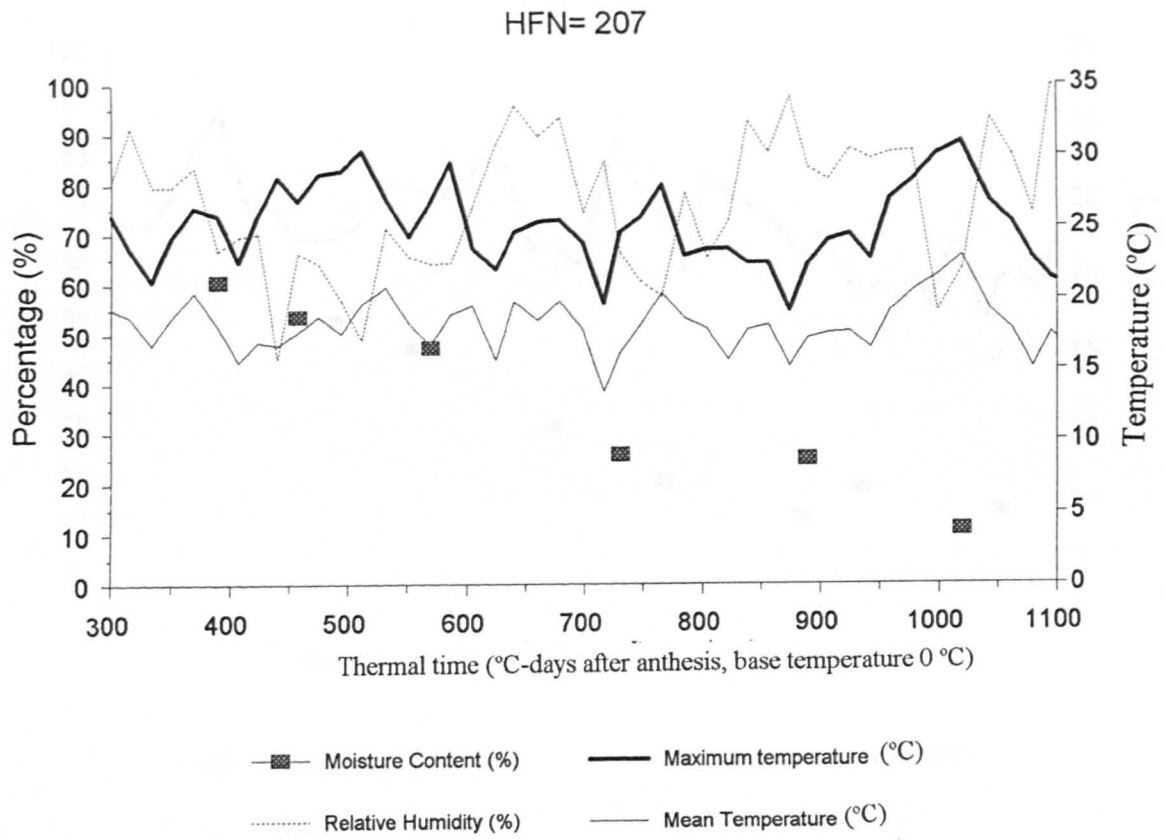


Figure 5.4 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at SB in 1994, where no PMAA was detected.

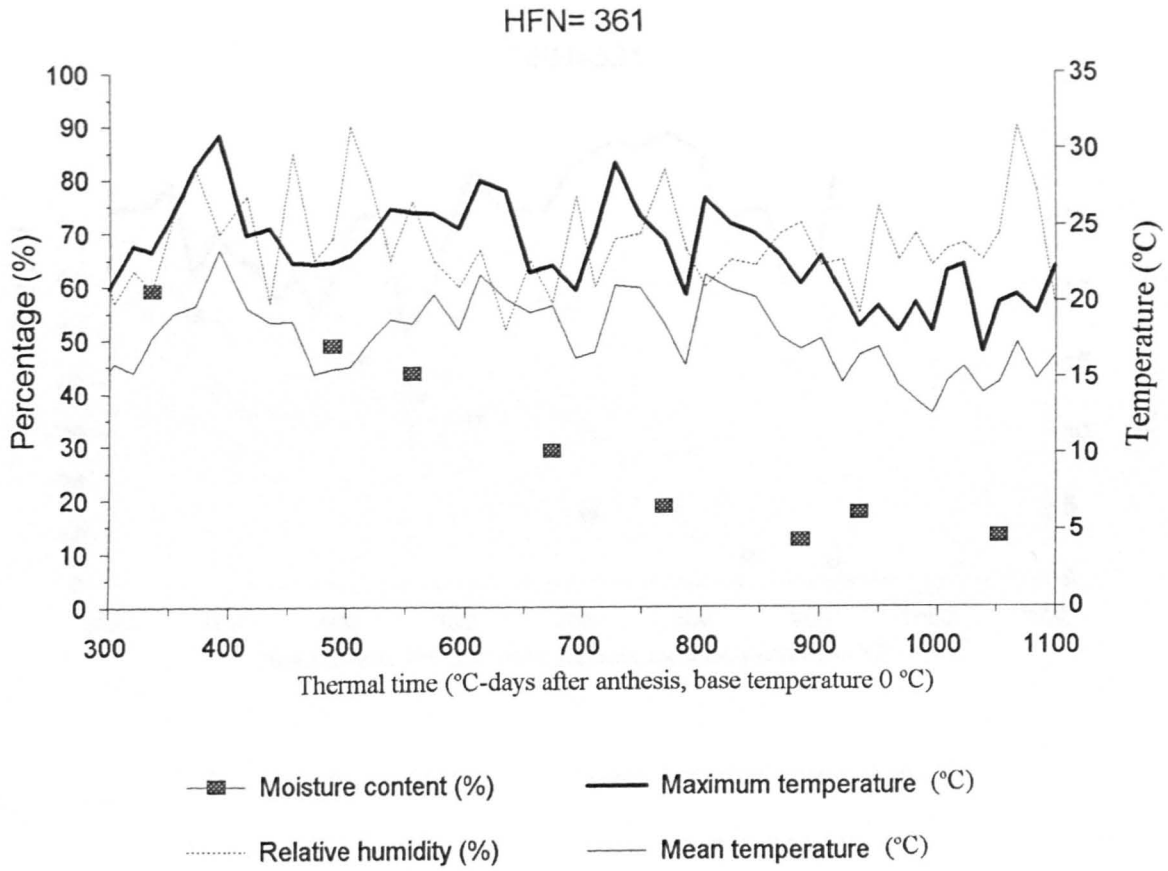


Figure 5.5 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at HA in 1995, where no PMAA was detected

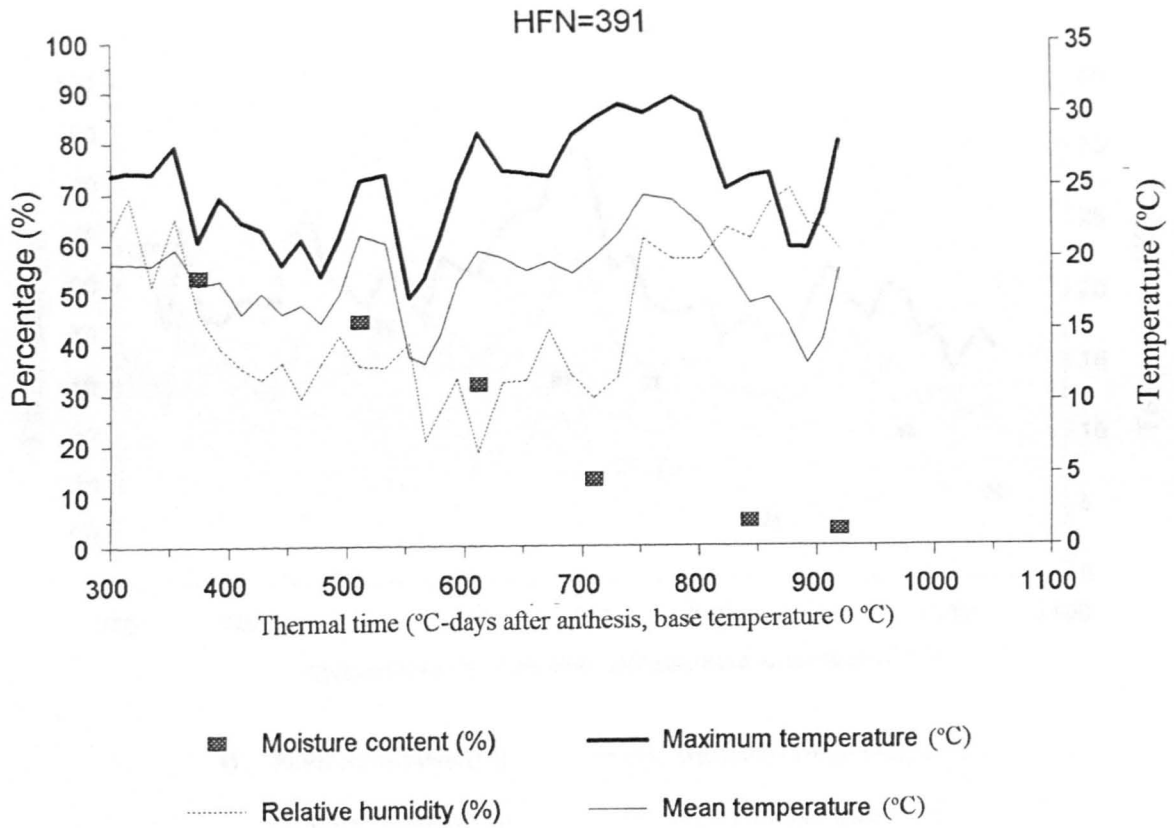


Figure 5.6 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at HA in 1996, where no PMAA was detected.

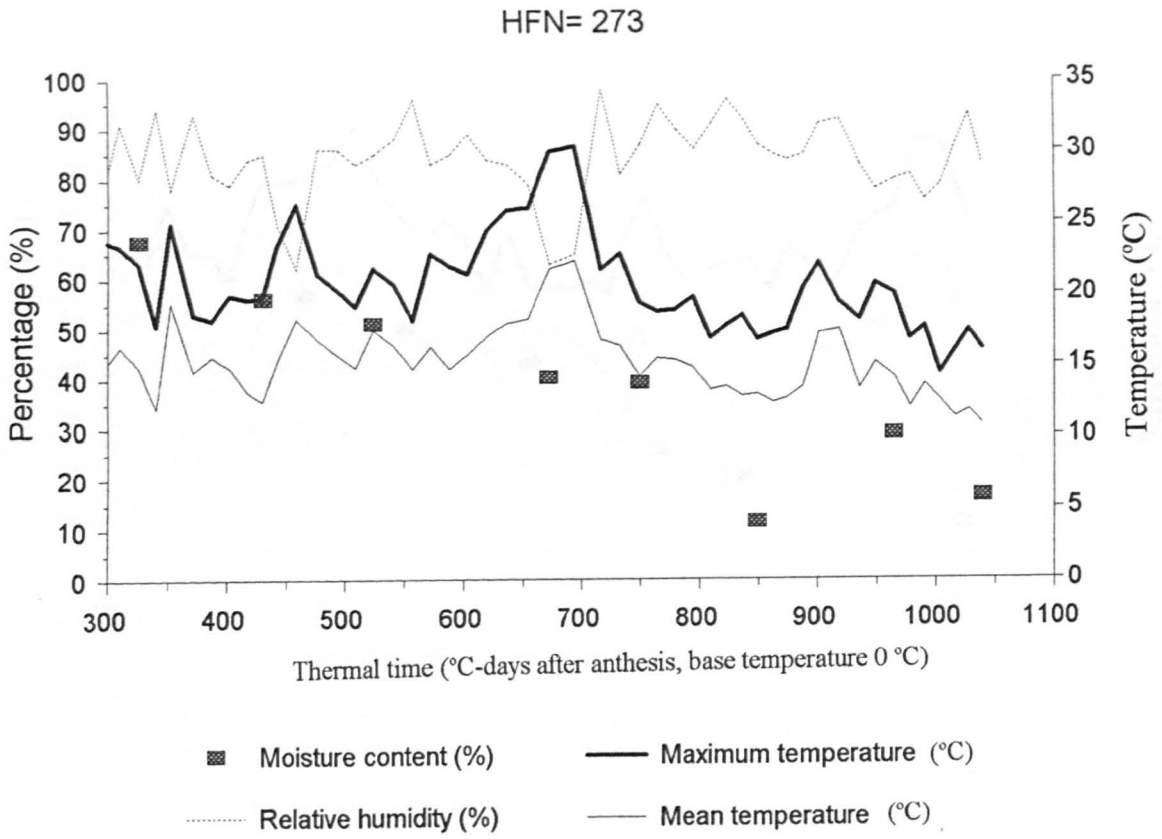
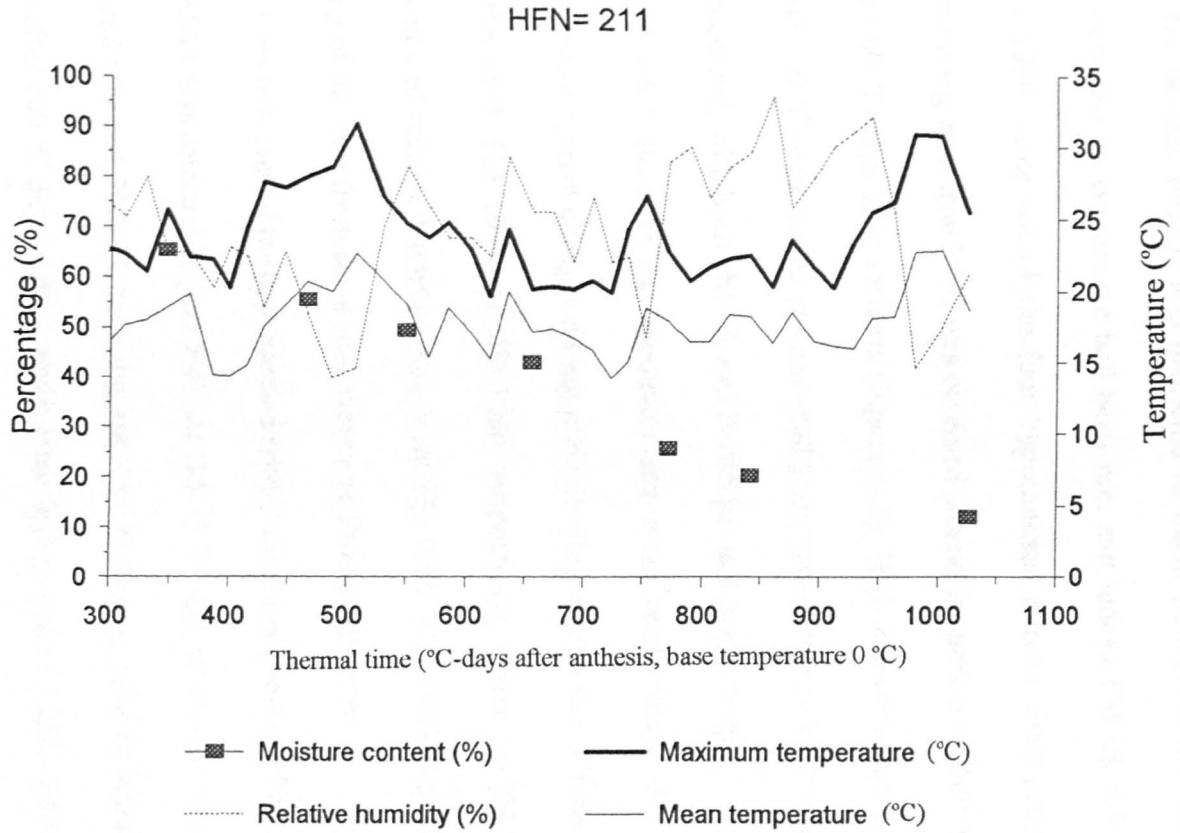


Figure 5.7 : The moisture content, maximum and mean temperature and relative humidity changes during grain development of the cultivar Hornet at SB in 1996, where no PMAA was detected.



therefore satisfied, and indeed PMAA was present. At AB 1995, maximum temperature greater than 25 °C was observed in the period between 600 and 700 °C-days after anthesis (Figure 5.2). A period of high humidity between 700 and 800 °C-days also occurred, with a grain drying-rate of 2.44% moisture loss day^{-1} measured. PoMS was also present in the sample. PMAA was predicted to occur as three out of the four hypothesised criteria for its occurrence had been met and indeed PMAA did occur. Similarly at AB 1996, three out of the four hypothesised criteria were present. A maximum temperature greater than 25 °C was observed around the period of 500 °C-days and just before 600 °C-days after anthesis (Figure 5.3). High humidity was apparent between 600 and 700 °C-days, however an overall grain drying-rate of 2.42% moisture loss day^{-1} was recorded, with both PMAA and PoMS present in the sample.

Figures 5.4-5.7 illustrate the maximum and mean temperature, the relative humidity and moisture content changes during grain development of the cultivar Hornet at SB 1994, HA 1995, HA 1996 and SB 1996 respectively, where no PMAA was detected. Absence of relative humidity data from SB 1995, UA 1995 and UA 1996 prevent plotting of the data from these sites where no PMAA occurred.

At SB 1994 only one of the hypothesised criteria for the presence of PMAA were met, and no PMAA was detected (Figure 5.4). At HA 1995 high maximum temperatures occurred around 600 and 700 °C days (Figure 5.5). However, relative humidity was relatively low after 600 °C days, with a rapid grain drying-rate of 3.25% moisture loss day^{-1} recorded due to the high mean temperatures. PMAA thus had a low predicted likelihood of occurrence as only one of the hypothesised criteria was met and indeed no PMAA was detected. At HA 1996, high maximum temperatures were seen around 700 °C-days (Figure 5.6) with a high relative humidity after 700 °C days giving a grain drying-

rate of 1.93% moisture loss day^{-1}. There was a small amount of PoMS in the sample but no PMAA was identified, despite two of the hypothesised criteria for the presence of PMAA being met. The fact that a period of high humidity did not directly follow a period of high temperature and the absence of high maximum temperatures earlier in grain development may explain the lack of appearance of PMAA. At SB 1996 only one of the four hypothesised criteria for the occurrence of PMAA was met (Figure 5.7). Thus, the predicted likelihood of PMAA occurring was small, and in fact no PMAA was detected.

Retrospective assessment of the likely occurrence or absence of PMAA in Hornet was assigned in seven out of the ten possible case scenarios examined from the field experiments (Table 5.1). The hypothesis generated from the controlled environment experiments could be used to justify the presence or absence of PMAA in Hornet in all of the seven cases.

5.3.2 Occurrence of PMAA in Riband

There was little difference in the developmental rates of Hornet and Riband (Table 3.14) at the seven case scenarios where predictions were possible. Hence the environmental conditions experienced by the two cultivars were similar, thus leading to a similar predicted occurrence or absence of PMAA in most cases. The occurrence of two cases of PMAA could be justified with the controlled environment experiments (Table 5.2). However, the occurrence of PMAA at AB 1996 was slightly surprising, The absence of high humidity conditions after a high maximum temperatures between 500 and 600 °C-days after anthesis suggested PMAA was unlikely. However, PMAA was identified suggesting that the early high temperature difference plays a more dominant role in determining PMAA, than high maximum temperature differences later in development.

Table 5.1 : Retrospective comparison between results obtained in the field experiments for the cultivar Hornet with the hypothesis generated from the controlled-environment cabinet experiments.

Site / Year	Grain development stages susceptible to transient changes in temperature					Predicted	Actual	
	500 °C-days → 600 °C-days 60% moisture content → 40% moisture content →				Grain drying-rate [a]			
	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	(% moisture loss day $^{-1}$)	Occurrence of PMAA	Occurrence of PMAA	Harvest HFN (s)
HA 1994	✓	✓	✓	✓	1.92	Yes	Yes	146
SB 1994	✗	✗	✓	✗	2.47	No	No	361
HA 1995	✗	✗	✓	✗	3.05	No	No	391
SB 1995	✗	?	✗	?	1.82	?	No	336
AB 1995	✗	✓	✓	✓	2.44	Yes	Yes	226 [b]
UA 1995	✗	?	✓	?	1.69	?	No	- [b]
HA 1996	✗	✓	✓	✗	1.93	No	No	273 [b]
SB 1996	✓	✗	✗	✗	2.11	No	No	211 [b]
AB 1996	✓	✗	✓	✓	2.42	Yes	Yes	207 [b]
UA 1996	✗	?	✗	?	1.18	?	Yes	117 [b]

✓ - Conditions apparent, ✗ - Conditions absent, ?- Not recorded, [a] Grain drying-rate data (40-20% grain moisture content) from Table 3.15, [b] - PoMS

Table 5.2 : Retrospective comparison between results obtained in the field experiments for the cultivar Riband with the hypothesis generated from the controlled-environment cabinet experiments.

Site / Year	Grain development stages susceptible to transient changes in temperature				Grain drying-rate [a]	Predicted	Actual	
	500 °C-days → 600 °C-days 60% moisture content → 40% moisture content →							
	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	(% moisture loss day $^{-1}$)	Occurrence of PMAA	Occurrence of PMAA	Harvest HFN (s)
HA 1994	✓	✓	✓	✓	1.83	Yes	Yes	288
SB 1994	✗	✗	✓	✗	2.11	No	No	321
HA 1995	✗	✗	✓	✗	2.60	No	No	376
SB 1995	✗	?	✗	?	2.72	?	No	348
AB 1995	✗	✓	✓	✓	1.79	Yes	Yes	243
UA 1995	✗	?	✓	?	1.57	?	No	- [b]
HA 1996	✗	✓	✗	✗	2.00	No	No	288 [b]
SB 1996	✓	✗	✗	✗	2.28	No	No	150 [b]
AB 1996	✓	✗	✗	✓	2.70	No	Yes	245 [b]
UA 1996	✗	?	✗	?	1.18	?	Yes	236 [b]

✓ - Conditions apparent, ✗ - Conditions absent, ?- Not recorded, [a] Grain drying-rate data (40-20% grain moisture content) from Table 3.15, [b] - PoMS

Table 5.3 : Retrospective comparison between results obtained in the field experiments for the cultivar Haven with the hypothesis generated from the controlled-environment cabinet experiments.

Site / Year	Grain development stages susceptible to transient changes in temperature					Predicted	Actual	
	500 °C-days → 600 °C-days 60% moisture content → 40% moisture content →				Grain drying-rate [a]			
	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	(% moisture loss day $^{-1}$)	Occurrence of PMAA	Occurrence of PMAA	Harvest HFN (s)
HA 1994	✗	✓	✓	✓	1.98	Yes	No	183 [b]
SB 1994	✗	✗	✓	✗	2.56	No	No	327
HA 1995	✗	✗	✓	✗	3.41	No	No	352
SB 1995	✗	?	✗	?	2.16	?	No	339
AB 1995	✗	✓	✓	✓	3.18	Yes	Yes	227 [b]
UA 1995	✗	?	✓	?	1.58	?	No	- [b]
HA 1996	✗	✓	✓	✗	1.93	No	No	253 [b]
SB 1996	✓	✗	✗	✗	3.24	No	No	239 [b]
AB 1996	✓	✗	✓	✓	2.49	Yes	Yes	203 [b]
UA 1996	✗	?	✗	?	1.24	?	Yes	181 [b]

✓ - Conditions apparent, ✗ - Conditions absent, ?- Not recorded, [a] Grain drying-rate data (40-20% grain moisture content) from Table 3.15, [b] -PoMS

Table 5.4 : Retrospective comparison between results obtained in the field experiments for the cultivar Pastiche with the hypothesis generated from the controlled-environment cabinet experiments.

Site / Year	Grain development stages susceptible to transient changes in temperature					Predicted	Actual	
	500 °C-days → 600 °C-days 60% moisture content → 40% moisture content →				Grain drying-rate [a] (% moisture loss day $^{-1}$)			
	High maximum temperature (> 25 °C)	High relative humidity (> 85%)	High maximum temperature (> 25 °C)	High relative humidity (> 85%)		Occurrence of PMAA	Occurrence of PMAA	Harvest HFN (s)
HA 1994	✓	✓	✓	✓	1.85	Yes	No	439
SB 1994	✗	✗	✓	✗	2.16	No	No	447
HA 1995	✗	✗	✓	✗	2.98	No	No	427
SB 1995	✗	?	✗	?	2.01	?	No	401
AB 1995	✗	✓	✓	✓	2.16	Yes	No	345
UA 1995	✗	?	✓	?	1.41	?	No	- [b]
HA 1996	✗	✓	✓	✗	1.96	No	No	356
SB 1996	✓	✗	✗	✗	2.44	No	No	357
AB 1996	✓	✗	✗	✓	2.83	No	No	418
UA 1996	✗	?	✗	?	1.14	?	No	328

✓ - Conditions apparent, ✗ - Conditions absent, ?- Not recorded, [a] Grain drying-rate data (40-20% grain moisture content) from Table 3.15, [b]-PoMS

5.3.3 Occurrence of PMAA in Haven

There was slightly more difference in the developmental rates of Hornet and Haven (Table 3.14) at the seven case scenarios where predictions were possible. Hence, the environmental conditions experienced by the two cultivars at set stages in development varied slightly. Of the three cases where PMAA was apparent, its occurrence could be justified with the controlled-environment cabinet experiment results in two cases. However, at HA 1994, PMAA did not occur in Riband despite seemingly favourable conditions for its occurrence. The only difference between the conditions experienced by Haven-HA-1994 and Hornet-HA-1994, in which PMAA did occur, was the absence of high temperatures between 500-600 °C-days after anthesis, caused by Haven reaching anthesis later (Table 5.3). This again suggests that a high temperatures at 500-600 °C-days after anthesis are particularly important in determining PMAA.

5.3.4 Occurrence of PMAA in Pastiche

No PMAA was identified in Pastiche in any of the field experiments (Table 5.4). This was despite conditions at HA 1994 and AB 1995 which suggested a high likelihood of its occurrence. This suggests that very specific conditions are required for Pastiche to generate PMAA in the field.

5.4 Discussion

The retrospective assessment of the likely occurrence of PMAA was correctly assigned in seven of the eight case scenarios examined from the field experiments where PMAA actually occurred, although a quantitative prediction of PMAA and HFN was not possible. The one exception, where PMAA was predicted to occur but did not, was Haven-HA-1994. In three other cases (Pastiche-HA-1994; Pastiche-AB-1995; Riband-AB-1995), PMAA was predicted to occur but was not detected. In both Haven-HA-1994

and Riband-AB-1995 the cause of error appeared to be related to the respective absence or occurrence of large temperature differences around 500-600 °C-days anthesis. This suggests that large temperature differences at this stage in grain development play a major role in determining the presence of PMAA. The cultivar Pastiche seems to require very specific conditions for high PMAA to be stimulated as no PMAA was detected in Pastiche grown in the field experiments (Section 3), whereas it was identified in the controlled environment experiments (Section 4). In the cultivar Hornet the absence of high temperatures early in grain development at HA-1996, could account for the absence of PMAA and the higher than expected HFN at this site, given that the grain drying-rate was similar to that at HA-1994 where PMAA was present and the harvest HFN was only 146 s. It also appears that mean temperature plays an important role in dictating the amount of PMAA. The high mean temperature after 800 °C days for Hornet-AB-1995, led to a grain drying-rate of 2.44% moisture loss day^{-1} and probably prevented the HFN from falling to a lower value than 226 s. High temperatures during the latter stages of grain development have been shown to be able to suppress PMAA in other studies as well (Mares and Gale, 1990). These results support the hypothesis that moisture availability during grain drying from 40-20% moisture content is an important factor in determining the amount of PMAA observed in the grain (Gold and Duffus, 1996). Further support for this hypothesis is provided by studies in Finland on spring wheat cultivars. This has shown that if the average relative humidity was below 70% and average maximum temperature exceeded 16 °C, during grain filling period, then the harvest HFN was greater than 230 s, the Finnish breadmaking HFN standard (Karvonen *et al.*, 1991). In contrast, when relative humidity was high (> 80%) and the mean maximum daily temperature was below 13 °C during grain filling, then the HFN fell below the Finnish commercially

acceptable standard of 120 s (Karvonen *et al.*, 1991).

This comparison between field experimental results (Section 3) and the controlled-environment cabinet results (Section 4) supports the hypothesis that a short period of high temperature at specific stages in grain development can enhance PMAA when coupled with conditions which produce a slow grain drying-rate.

The concurrence between the results from the field experiments and controlled environment experiments was encouraging in respect to the qualitative assessment of the occurrence of PMAA. These results however, require verification using further data sets. The quantification of the effect of transient changes in temperature and grain drying-rate on PMAA requires a great deal of further work, if an accurate quantitative prediction of PMAA and HFN is to be made on the basis of meteorological variables. Obtaining the degree of accuracy from a meteorological-based HFN prediction scheme, far enough in advance of harvest to enable growers to significantly alter their harvest management plans, may well be hard to achieve. An empirical study using H-GCA HFN quality survey data from 57 cultivars between 1975-1995, has shown that annual variation in HFN can be accounted for by meteorological data (maximum and minium temperature and rainfall), but not until late in the season i.e. not until the beginning of August did the percentage variance accounted for rise above 70%, reaching 85% at harvest time (Smith and Gooding, 1999). This again suggests that growers would have little time to alter their harvest management strategy on the basis of a meterological-based HFN prediction scheme.

Recent work has shown about an 8 year cyclical pattern in mean national HFN ($P=0.002$) in England and Wales. This in turn has been shown to have a positive relationship with the mean of the January and February North Atlantic Oscillation (NA0)

index (Kettlewell *et al.*, 1999). The NAO index is a measure of the atmospheric pressure difference over the Atlantic Ocean between the Azores and Iceland. A negative relationship between the NAO index and August rainfall was also identified ($P<0.008$), suggesting the effects of NAO on HFN are largely due to the influence of rainfall on PoMS. The NAO index was also shown to be related to the price difference between feed and breadmaking wheats and the amount of wheat imported to the UK. This relationship between a meteorological variable approximately five months before harvest and HFN, could be useful to importers and millers allowing them a forewarning of the likely overall HFN quality of the UK wheat crop. This would enable them to plan the sourcing of possible alternate supplies of high HFN wheat. This early meterological-based HFN prediction would be of little practical use to individual growers wishing to know the specific HFN of their individual crops at harvest. However, the identified cyclical pattern in national HFN does provide growers with some indication of the likely overall HFN of the following years crop. This could allow growers to make a better assessment of the risk and associated benefits of sowing feed or breadmaking wheats in certain years. The development of an accurate meterological-based HFN prediction scheme to account for all the effects of weather on *alpha*-amylase activity during grain development, still presents a formidable challenge.

6. General Discussion

Using data from the field experiments and controlled-environment cabinet experiments it was hoped a parsimonious prediction scheme for high PMAA and low HFN could be devised. The development of an uncomplicated prediction scheme which conservatively uses data to produce accurate HFN predictions has been thwarted by the variability in the results obtained in the experiments and the complexity of environmental interactions on PMAA. Nonetheless the results have enabled several factors to be identified as key components in a scheme hoping to predict the occurrence of PMAA and the HFN of harvest ripe grain.

1) This thesis has shown that a wide variation in PMAA is produced by different cultivars in both field experiments (Section 3) and controlled-environment cabinet experiments (Section 4), illustrating that the susceptibility of a cultivar to PMAA must be included in an HFN prediction scheme. The unexpected occurrence of high PMAA in Pastiche in the controlled-environment cabinet experiments shows that PMAA can be induced in cultivars not recognised as sensitive to the formation of high PMAA from the national ratings (Anon., 1991). These ratings should therefore not be relied upon to determine, with certainty, the susceptibility of current cultivars to PMAA.

2) This thesis has also illustrated the relative importance of the four routes causing high *alpha*-amylase activity in UK wheat crops. PMAA, was identified as the sole cause of low HFN in 2 of the 18 site x year x cultivar combinations where the HFN fell below 250 s and as a contributory cause in a further eight cases. It was shown to play a more important role than either PrMS or RPAA, which were seen in isolated grains in some samples but did not occur with enough severity to decrease HFN to below 250 s. PoMS was the dominant cause of low HFN occurring in 16 of the 18 site x year x cultivar

combinations where the HFN fell below 250 s. These results have highlighted that the occurrence of PMAA must not be overlooked by cereal breeders in their breeding selection programmes where sprouting resistance to PoMS is primarily selected for rather than low *alpha*-amylase activity (Gale, 1989). Results from the field experiments have also shown that it is sometimes difficult to assign one route as the sole cause of high *alpha*-amylase activity and low HFN. In a large scale commercial field it is possible that a sample with a low HFN could be caused by a combination of PMAA and PoMS, with RPAA and PrMS also acting as minor contributory factors. The actual level of influence of each of these routes on the final HFN will make it difficult to give an accurate quantitative prediction of HFN.

3) This thesis has shown that slow grain drying-rates between 40-20% grain moisture content are associated with higher PMAA and lower HFN, than a fast grain drying-rate, supporting the hypothesis of Gale *et al.* (1983). The actual quantification of the effect of grain drying-rate on PMAA is more problematic due to the occurrence of PoMS in many of the samples. PMAA has been found associated with grain drying-rates as high as 3.18% moisture loss day $^{-1}$ (AB 1995 - Haven), although the actual amount of PMAA in this case is hard to deduce as PoMS also occurred. In cases were PMAA solely occurred (HA 1994 - Hornet) and (AB 1995 - Riband) grain drying-rates were 1.92% and 1.79% moisture loss day $^{-1}$ respectively which were both greater than grain drying-rates previously thought to prevent the expression of PMAA.

4) This thesis has established a sampling time point (850 °C-days / 35% moisture / ZGS 85-87) when a pre-harvest HFN sample can be taken which will accurately predict the actual combine-harvest HFN in the absence of PoMS. This sampling time point will identify the initial occurrence of any PMAA in the grain, which has been identified as

starting to accrue in the grain from around a grain moisture content of 47.8 %. Subsequent slow grain drying conditions may lead to an increase in PMAA and this is probably the cause of the wide confidence limits associated with the HFN prediction. It is also possible that RPAA at pre-harvest sampling may be causing the wide confidence limits as this may completely degrade before combining. A later pre-harvest HFN sample would increase the accuracy of the HFN predictions but would give growers less time to implement a harvest management strategy (Kettlewell, 1993).

5) This thesis has aided in the development of a pre-harvest HFN prediction scheme (Lunn *et al.*, 1998), with the HFN sampling time point, the actual HFN sampling strategy and the transport and processing of HFN samples all evaluated (Section 3).

I) An earliest sampling time point when a pre-harvest HFN sample can be taken has been established at 850 °C-days (35% moisture / ZGS 85-87).

ii) Pre-harvest sampling at four points from within the plots, to generate a pre-harvest sample of 350-400 ears, was shown to give the most precise HFN measurement.

iii) Transport of samples by post for next day delivery to a central site for processing was shown to be feasible. Centralised processing of fresh ear samples gave more consistent results than the drying of samples at diverse sites and subsequent dispatch of samples for processing, illustrating drying of pre-harvest HFN samples must be carefully monitored.

6)This thesis has identified that large transient increases in temperature in the grain during the early stages of grain development (Section 4) are capable of stimulating subsequent high PMAA if high humidity / slow grain drying conditions are apparent. This effect was however found not to be consistent suggesting a role of other environmental factors in affecting PMAA. This identification of an early environmental stimulus for high

PMAA, may explain some of the inconsistencies regarding the relationship between the subsequent grain drying-rate between 40-20% grain moisture content and PMAA. High temperature changes and any other environmental factors identified as stimulating PMAA must therefore be incorporated into any agrometeorologically-based HFN prediction scheme.

7) The analysis of a comparison between the field and controlled-environment experimental results (Section 5) highlights that after favourable conditions for the stimulation of PMAA, subsequent environmental factors affecting moisture relationships and grain drying-rate, will affect PMAA supporting the hypothesis of Gold and Duffus (1996). Factors affecting grain drying-rate would thus also have to be included in an agrometeorologically-based HFN prediction scheme.

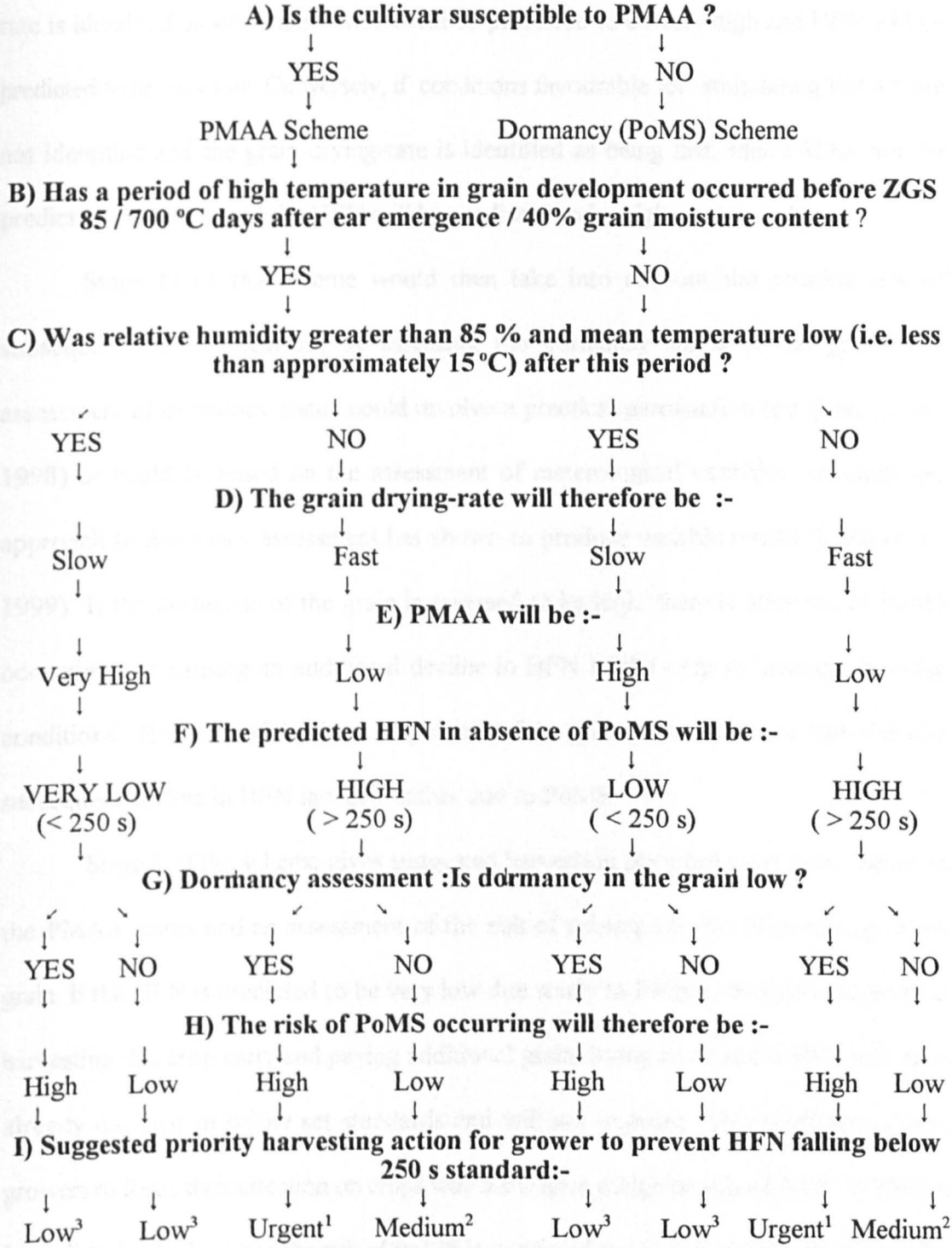
Even if these interactions between grain physiology and environmental factors are all deduced, the success of a HFN prediction scheme, will still largely depend on the accuracy of weather forecasts made by meteorologists and the faith put in them by growers. In the overall group project the complexity of relationships identified between temperature and dormancy has also precluded the development of an agrometeorologically based scheme for predicting HFN and sprouting (Lunn *et al.*, 1998). Instead a simplified scheme based on pre-harvest sampling and assessment of dormancy has been devised to allow a practical prediction scheme to operate (Kettlewell *et al.*, 1998). This simplified HFN prediction scheme was tested in 1997 and predictions proved to be correct in thirty-five out of the forty predictions made *i.e.* 85% (Lunn *et al.*, 1998). The main errors in the predictions were thought to be due to the increased amount of PMAA which accrued as the grain dried out and was not fully accounted for in the pre-harvest sampling measurement, i.e. the predicted HFN was higher than that obtained at harvest.

Greater understanding of the role of water relationships within the grain during grain drying are required for any successful modelling of late increases in PMAA as the grain approaches combinable harvest ripeness to be achieved. Figure 6.1 illustrates the stages involved in a simplified scheme for predicting the occurrence of high PMAA and a low harvest HFN.

Stage A of the prediction scheme would involve an assessment of the cultivars genetic susceptibility to the formation of high PMAA. Currently this assessment would be based on a simple hereditary analysis of the cultivar to identify the possible presence of any known PMAA susceptible cultivars in its lineage (e.g. See Table 2.5). If these are present in the lineage of the cultivar it would be included in the PMAA scheme. Alternatively a screening tool may be developed allowing a rating for PMAA susceptibility to be assigned to new cultivars, in a similar way to which NIAB rates PoMS (McVittie and Draper, 1982a ;Anon, 1991). Cultivars non-susceptible to high PMAA would enter directly into a scheme assessing the dormancy and PoMS resistance of the cultivar (Stage D).

Cultivars identified as susceptible to PMAA would enter into Stage B of the scheme. This requires an assessment of whether high temperatures early in grain development (before ZGS 85 / 700 °C days after ear emergence / 40% grain moisture content), which can stimulate high PMAA, have occurred. Other environmental conditions which may also stimulate high PMAA could also subsequently be included in the scheme at this stage. The actual assessment would be based on meteorological records and observations on the development of the crop. A simplified yes or no answer as to whether these conditions had occurred could be made. This then leads onto Stage C of the scheme which requires an assessment of grain drying-rate, either directly by grain moisture

Figure 6.1 : Simplified diagram of the stages in a potential prediction scheme for the occurrence of high PMAA and low HFN.



[1] If the weather forecast is unfavourable harvest the crop at a high moisture content (e.g. 20%) and employ grain drying to ensure HFN remains high.
[2] Crops will show some resistance to a decline in HFN in wet weather.
[3] HFN already below acceptable standard, focus on harvesting other better quality crops first.

measurements or by using meterological records to extrapolate likely grain-drying rates. If conditions stimulating high PMAA had been identified in Stage A and the grain drying-rate is identified as slow then PMAA will be predicted to be very high and HFN will be predicted to be very low. Conversely, if conditions favourable for stimulating PMAA are not identified and the grain drying-rate is identified as being fast, then PMAA will be predicted to be low, and the HFN will be predicted to be high.

Stage D of the scheme would then take into account the possible risk of subsequent PoMS occurring by assessing the dormancy status of the grain. The assessment of dormancy status could involve a practical germination test (Lunn *et al.*, 1998) or could be based on the assessment of meterological variables, although this approach to dormancy assessment has shown to produce variable results (Lunn *et al.*, 1999). If the dormancy of the grain is assessed to be high, there is little risk of PoMS occurring and causing an additional decline in HFN of the crop in 'average growing conditions'. However, if the dormancy status of the grain is low there is a high risk of a subsequent decline in HFN in wet weather due to PoMS.

Stage E of the scheme gives suggested harvesting priorities to growers based on the PMAA status and an assessment of the risk of subsequent PoMS occurring in the grain. If the HFN is predicted to be very low due solely to PMAA, there is little point in harvesting this crop early and paying additional grain drying costs as the HFN will have already declined to below set standards and will not improve. This prediction allows growers to focus their attention on crops which still have a high predicted HFN. If PMAA is predicted to be low, and the risk of PoMS is predicted to be high and the forecast is for wet weather, then harvesting the crop at a high moisture content and employing grain driers will ensure the HFN remains high. The decision to harvest earlier will also have to

consider the additional grain drying costs incurred, and the likely market value of high HFN grain. If PMAA and the risk of PoMS is predicted to be low, and the forecast is for wet weather, then growers may opt to leave this crop in the field and focus attention on harvesting crops at high risk of PoMS and a decline in HFN first. Alternatively growers may harvest crops with a high predicted HFN and low PoMS resistance first, to guarantee their store contains some valuable high HFN grain.

This scheme gives growers additional information as to the likely HFN of their crop allowing them to plan the harvest management of their crops with more certainty. Individual growers faith in weather forecasts and their perception of risk will determine how they actually utilise the HFN predictions.

6.1 Future work

A great deal of further investigation on the effects of environmental factors on PMAA is required if this phenomenon is to be fully understood and an accurate agrometeorological model established for the prediction of HFN. The precise stage in grain development which is susceptible to high PMAA has not been defined in these studies and requires further characterization. In the controlled-environment cabinet experiments only the effect of one transient increase or decrease in temperature during grain development was considered. In the field environment several periods of large temperature fluctuations are more likely to occur. Further investigation of the effects of several changes in temperature on PMAA are required. Additionally further studies are required to identify other possible stages in grain development which may be susceptible to the development of high PMAA due to transient temperature changes. The actual magnitude of the temperature change which stimulates enhanced PMAA also requires

more accurate quantification. Controlled-environment cabinet experiment, treatments were based on constantly maintained temperature regimes which are very different from the variable temperature regimes likely to be experienced in the field environment.

In this experimental work temperature has been considered as the principal factor determining PMAA in the grain. The large differences in *alpha*-amylase activity between experiments in the controlled-environment cabinets suggests other possible environmental stimuli also require investigation to establish the importance of their roles in determining PMAA in the grain. The effects of humidity on PMAA would appear to be the next most logical environmental factor to investigate, particularly as other recent studies (Nakatsu, 1999) have also implicated it as playing a key role in determining PMAA.

Grain physiological studies also need to be undertaken to investigate the role of environmental temperature on the sensitivity of immature aleurone tissue to GA, to see if this offers an explanation as to the effect of transient changes in temperature on PMAA. Greater understanding of the role of sucrose, grain water relations, hormonal physiology, membrane structure and genetic regulation of *alpha*-amylase expression would also help in the elucidation of the mechanism and causative effects leading to high PMAA in the grain.

Extrapolating the controlled-environment experiment results to develop an accurate agrometeorological based prediction scheme will be not be an easy task. In this work the same cultivars as used in the field experiments were used in the controlled-environment experiments. It was hoped that this would enable comparisons between the two environments to be made, enabling factors stimulating high PMAA to be isolated and further investigated. On hindsight it may have been preferable to use cultivars which have already been shown to be highly susceptible to high PMAA (e.g. Spica, BD-159), allowing

the effects to be more easily identified. The problem with this approach is that the results may not accurately reflect what is likely to occur in currently grown UK cultivars.

In the long term it is plant breeders who hold the key to the problem of low HFN. They have been significantly improving the HFN of UK wheat cultivars with about a 40 s improvement in HFN identified in cultivars grown in the 1990's compared to their predecessors grown in the 1970's (Smith and Gooding, 1999).

If a practical HFN prediction scheme is to be developed, data on the responsiveness of current commercial cultivars to the environment and PMAA needs to be gathered, to allow accurate predictions to be made and a large enough database for an "agrometeorological" model for high PMAA to be established. The predictions will especially need to be fine tuned around the 250 s HFN specification as it is here that commercial considerations are most critical.

A project seeking to resolve some of these issues has recently been instigated : *Practical tests of a prototype scheme for pre-harvest prediction of Hagberg falling number in wheat.* This 6 month project funded by the H-GCA (Project No. 192) involves liaison between Harper Adams University College, University of Nottingham, University of Aberdeen, ADAS-Bridgets, NIAB Labtest ® and several members of the Association of Independent Crop Consultants (AICC). It is hoped this project will allow a commercial HFN prediction scheme service based on pre-harvest crop sampling for HFN to be established for use in 1999.

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Refereed papers in preparation

Lunn, G.D., Kettlewell, P.S., Scott, R.K. and Major, B.J. (in preparation) Low Hagberg falling number in immature wheat grain, I : Pericarp *alpha*-amylase activity.

Major, B.J., Kettlewell, P.S., Lunn, G.D. and Scott, R.K. (in preparation) Low Hagberg falling number in immature wheat grain, II : Pre-maturity *alpha*-amylase activity.

Lunn, G.D., Scott, R.K., Kettlewell, P.S., Major, B.J. and Oakley, J.N. (in preparation) Low Hagberg falling number in immature wheat grain, III : Pre-maturity sprouting.

Appendix : 1

1A : Experimental layout of field experiment plots at Harper Adams University College 1993/1994.

BLOCK I

NOT MISTED		MISTED	
	Discard		Discard
16	Haven	17	Scipion
15	Pastiche	18	Hornet
14	Recital	19	Soissons
13	Hornet	20	Recital
12	Thesee	21	Thesee
11	Riband	22	Haven
10	Soissons	23	Riband
9	Scipion	24	Pastiche
	Discard		Discard

BLOCK II

MISTED		NOT MISTED	
	Discard		Discard
	Discard		Discard
8	Thesee	25	Recital
7	Hornet	26	Haven
6	Haven	27	Thesee
5	Pastiche	28	Pastiche
4	Scipion	29	Riband
3	Soissons	30	Hornet
2	Riband	31	Scipion
1	Recital	32	Soissons
	Discard		Discard

BLOCK III

MISTED		NOT MISTED	
	Discard		Discard
	Discard		Discard
40	Scipion	41	Pastiche
39	Thesee	42	Riband
38	Soissons	43	Soissons
37	Recital	44	Thesee
36	Haven	45	Haven
35	Riband	46	Hornet
34	Pastiche	47	Recital
33	Hornet	48	Scipion
	Discard		Discard

East
North ◂---|--- South
West

1B : Experimental layout of field experiment plots at Harper Adams Agricultural College 1994/1995

	Discard	Discard	Discard	Discard	
	29a Recital	25a Hornet	5a Scipion	1a Riband	
	29b Recital	25b Hornet	5b Scipion	1b Riband	
BLOCK I	30a Scipion	26a Riband	6a Recital	2a Hornet	BLOCK I
	30b Scipion	26b Riband	6b Recital	2b Hornet	
MISTED	31a Thesee	27a Haven	7a Thesee	3a Haven	NOT MISTED
	31b Thesee	27b Haven	7b Thesee	3b Haven	
	32a Soissons	28a Pastiche	8a Soissons	4a Pastiche	
	32b Soissons	28b Pastiche	8b Soissons	4b Pastiche	
	Discard	Discard	Discard	Discard	
	Discard	Discard	Discard	Discard	
	9a Recital	13a Hornet	33a Thesee	37a Haven	
	9b Recital	13b Hornet	33b Thesee	37b Haven	
BLOCK II	10a Thesee	14a Haven	34a Soissons	38a Pastiche	BLOCK II
	10b Thesee	14b Haven	34b Soissons	38b Pastiche	
NOT MISTED	11a Soissons	15a Riband	35a Recital	39a Hornet	MISTED
	11b Soissons	15b Riband	35b Recital	39b Hornet	
	12a Scipion	16a Pastiche	36a Scipion	40a Riband	
	12b Scipion	16b Pastiche	36b Scipion	40b Riband	
	Discard	Discard	Discard	Discard	
	Discard	Discard	Discard	Discard	
	45a Thesee	41a Haven	17a Scipion	21a Riband	
	45b Thesee	41b Haven	17b Scipion	21b Riband	
BLOCK III	46a Recital	42a Hornet	18a Recital	22a Pastiche	BLOCK III
	46b Recital	42b Hornet	18b Recital	22b Pastiche	
MISTED	47a Soissons	43a Pastiche	19a Thesee	23a Hornet	NOT MISTED
	47b Soissons	43b Pastiche	19b Thesee	23b Hornet	
	48a Scipion	44a Riband	20a Soissons	24a Haven	
	48b Scipion	44b Riband	20b Soissons	24b Haven	
	Discard	Discard	Discard	Discard	

South

East ◂---|--- West

North

1C : Experimental layout of field experiment plots at Harper Adams University College 1995/1996

BLOCK III	BLOCK II	BLOCK I
NOT MISTED	MISTED	NOT MISTED

Discard	Discard	Discard	Discard	Discard	Discard
17a Hornet	21a Recital	37a Scipion	33a Riband	5a Scipion	1a Hornet
17b Hornet	21b Recital	37b Scipion	33b Riband	5b Scipion	1b Hornet
18a Riband	22a Soissons	38a Recital	34a Hornet	6a Thesee	2a Riband
18b Riband	22b Soissons	38b Recital	34b Hornet	6b Thesee	2b Riband
19a Haven	23a Thesee	39a Thesee	35a Haven	7a Soissons	3a Haven
19b Haven	23b Thesee	39b Thesee	35b Haven	7b Soissons	3b Haven
20a Pastiche	24a Scipion	40a Soissons	36a Pastiche	8a Recital	4c Pastiche
20b Pastiche	24b Scipion	40b Soissons	36b Pastiche	8b Recital	4c Pastiche
Discard	Discard	Discard	Discard	Discard	Discard
Discard	Discard	Discard	Discard	Discard	Discard
45a Recital	41a Hornet	9a Hornet	13a Recital	29a Thesee	25a Rband
45b Recital	41b Hornet	9b Hornet	13b Recital	29b Thesee	25b Riband
46a Soissons	42a Haven	10a Pastiche	14a Thesee	30a Soissons	26a Hornet
46b Soissons	42b Haven	10b Pastiche	14b Thesee	30b Soissons	26b Hornet
47a Scipion	43a Riband	11a Haven	15a Scipion	31a Recital	27a Pastiche
47b Scipion	43b Riband	11b Haven	15b Scipion	31b Recital	27b Pastiche
48a Thesee	44a Pastiche	12a Riband	16a Soissons	32a Scipion	28a Haven
48b Thesee	44b Pastiche	12b Riband	16b Soissons	32b Scipion	28b Haven
Discard	Discard	Discard	Discard	Discard	Discard

MISTED	NOT MISTED	MISTED
BLOCK III	BLOCK II	BLOCK I

East
North ◂---|--- South
West

Appendix : 2

2A : Crop Husbandry - Harper Adams University College
Field : Near Cot Leasow 1993/1994

Sown - 2nd November 1993

Nitrogen Application

Date	Product	Rate
26/3/94	NURAM (Urea ammonium nitrate)	40 kg N ha^{-1}
1/5/94	NURAM	60 kg N ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
18/4/94	CYCOCEL (Chlormequat+ Choline Chloride)	2.5 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
9/5/94	SANCTION (Flusilazole) CORBEL (Fenpropimorph)	0.4 l ha^{-1} 1.0 l ha^{-1}
21/5/94	FOLICUR (Tebuconazole)	1.0 l ha^{-1}
31/5/94	FOLICUR CORBEL ROVER 500 (Chlorothalonil)	0.5 l ha^{-1} 0.5 l ha^{-1} 1.0 l ha^{-1}

No Autumn or Spring Herbicides were applied.

2B : Crop Husbandry - Harper Adams University College
Field : Swans Leasow 1994/1995

Sown - 27 th October 1994

Seed bed Preparation - Ploughed, Power Harrowed.

Previous crop - Sugar Beet

Soil Status (Tested 7/3/95)

Soil Type	pH	K^+	Mg^{2+}	P	Residual Soil Nitrogen
Very slightly stony sandy loam	6.6	25 mg l^{-1}	94 mg l^{-1}	103 mg l^{-1}	54 kg ha^{-1}

Nitrogen and Manganese Application

Date	Product	Rate
7/3/95	Mineral Nitrogen determined by soil sampling	54 kg N ha^{-1}
13/3/95	Propol Manganese	1.0 l ha^{-1}
15/3/95	Ammonium nitrate prills	30 kg N ha^{-1}
20/4/95	Ammonium nitrate prills	99 kg N ha^{-1}
27/5/95	Ammonium Nitrate prills	75 kg N ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
21/3/95	CYCOCEL (Chlormequat+ Choline Chloride)	1.75 l ha^{-1}
13/4/95	CYCOCEL	0.75 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed Treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
13/3/95	BAS464 (Fenpropimorph + Tridemorph)	0.5 l ha^{-1}
21/3/95	SANCTION (Flusilazole)	0.4 l ha^{-1}
12/6/95	OPUS (Epoxiconazole)	1.0 l ha^{-1}

Herbicide Application.

Date	Product	Rate
21/3/94	IPU (Isoproturon) STOMP (Pendimethalin)	3.3 l ha^{-1}
13/4/95	HARMONY M (Metsulfuron-methyl + Thifensulfuron-methyl)	2.5 l ha^{-1}

Insecticide Application.

Date	Product	Rate
12/6/95	APHOX (Pirimicarb)	250 g ha^{-1}

2C : Crop Husbandry - Harper Adams University College
Field C : Birds Nest 1995/1996

Sown - 8 th November 1995

Seed bed Preparation - Ploughed, Power Harrowed.

Previous crop - Potatoes

Soil Status (Tested 4/3/95)

Soil type	pH	K^+	Mg^{2+}	P	Residual soil Nitrogen
Very slightly stony sandy loam	7.5	226 mg l^{-1}	152 mg l^{-1}	103.7 mg l^{-1}	120.7 kg ha^{-1}

Nitrogen and Manganese Application

Date	Product	Rate
20/3/96	NURAM (Urea ammonium nitrate)	60 kg N ha^{-1}
21/4/96	NURAM	100 kg N ha^{-1}
4/4/96	PROFOL MANGANESE	5 kg ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
15/4/96	NEW 5C CYCOCEL (Chlormequat + Choline Chloride)	1.75 l ha^{-1}
27/5/96	TERPAL (2-chloroethylphosphonic acid + mepiquat chloride)	2.0 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
6/5/96	SANCTION (Flusilazole) MISTRAL (Fenpropimorph)	0.5 l ha^{-1} 1.0 l ha^{-1}
2/7/96	BAS 464 (Fenpropimorph + Tridemorph)	1.0 l ha^{-1}
16/7/96	GRAPHIC (Fenbuconazole + propiconazole)	1.0 l ha^{-1}

Herbicide Application

Date	Product	Rate
23/11/95	JAVELIN GOLD (Diflufenican + Isoproturon)	5.0 l ha^{-1}
24/5/96	ALLY (Metsulfuron-Methyl) STARANE (Fluroxypyr))	30 g ha^{-1} 1.0 l ha^{-1}

2D : Crop Husbandry - Sutton Bonington- Field 2 : 1993/1994

Sown - 10 th November 1995

Seed bed Preparation - Ploughed, Power Harrowed.

Previous crop - Potatoes

Soil Status (Tested 4/3/95)

Nitrogen and Manganese Application

Date	Product	Rate
22/3/94	Mineral Nitrogen determined by soil sampling	63 kg N ha^{-1}
23/3/94	Ammonium nitrate prills	40 Kg N ha^{-1}
19/4/94	Ammonium nitrate prills	73 Kg N ha^{-1}
5/5/94	Ammonium nitrate prills	32 Kg N ha^{-1}
27/5/94	Cutonic Extra Mn	1.0 l ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
18/4/94	CHLORMEQUAT (Chlormequat)	2.3 l ha^{-1}
12/5/94	TERPAL (2-Chloroethylphosponic acid + mepiquat chloride)	2.0 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed Treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
30/4/94	SPORTAK 45 (Prochloraz)	0.9 l ha^{-1}
12/5/94	CORBEL (Fenpropimorph)	1.0 l ha^{-1}
27/5/94	FOLICUR (Tebuconazole)	1.0 l ha^{-1}
11/6/94	PATROL (Fenpropidin)	1.0 l ha^{-1}
30/6/94	SILVACUR (Tebuconazole + Triadimenol)	1.0 l ha^{-1}

Herbicide Application

Date	Product	Rate
21/3/94	PANTHER (Isoproturon + Diflufenican)	2.0 l ha^{-1}

Insecticide Application

Date	Product	Rate
30/6/94	APHOX (Pirimicarb)	280 g ha^{-1}

2G : Crop Husbandry - University of Aberdeen - Field : Botany : 1994/1995

Sown - 24 th October 1994

Nitrogen and Manganese Application

Date	Product	Rate
24/10/94	ON 90P 90K	-
23/3/95	Ammonium nitrate prills	40 Kg N ha^{-1}
27/4/95	Ammonium nitrate prills	100 Kg N ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
28/4/95	5-C CYCOCEL (Chlormequat + Choline Chloride)	2.5 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed Treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
12/5/94	SPORTAK DELTA (Prochloraz) PATROL (Fenpropidin)	1.25 l ha^{-1} 0.75 l ha^{-1}
6/6/95	FOLICUR (Tebuconazole) PATROL (Fenpropidin)	1.0 l ha^{-1} 0.75 l ha^{-1}
8/7/95	IMPACT EXCEL (Chlorothalonil + flutriafol)	2.0 l ha^{-1}

No Herbicide Application

2H : Crop Husbandry - University of Aberdeen - Field : Skillydams : 1994/1995

Sown - 18 th October 1995

Nitrogen and Manganese Application

Date	Product	Rate
18/10/95	ON 90P 90K	-
6/3/96	Ammonium nitrate prills	40 Kg N ha^{-1}
19/4/96	Ammonium nitrate prills	140 Kg N ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
8/5/96	5-C CYCOCEL (Chlormequat + Choline Chloride)	2.5 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed Treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
12/5/96	SPORTAK DELTA (Prochloraz + Cyproconazole) PATROL (Fenpropidin)	1.25 l ha^{-1} 0.75 l ha^{-1}
6/6/96	FOLICUR (Tebuconazole) PATROL (Fenpropidin)	1.0 l ha^{-1} 0.75 l ha^{-1}
8/7/96	IMPACT EXCEL (Chlorothalonil + flutriafol)	2.0 l ha^{-1}

Herbicide Application

Date	Product	Rate
29/4/96	ALLY (Isoproturon,Diflufenican) SWIPE (Bromoxynil + Ioxynil + Mecoprop-P)	15 g ha^{-1} 2.5 l ha^{-1}

2I : Crop Husbandry - ADAS Bridgets : Field : Nevada 1994/1995

Sown - 24 th October 1994

Seed bed Preparation - Ploughed and presse

Soil Status (Tested 1995)

Soil type	pH	K^+	Mg^{2+}	P	Residual soil Nitrogen
Silty clay loam	8.2	123 mg l^{-1}	36 mg l^{-1}	26 mg l^{-1}	-

Nitrogen and Manganese Application

Date	Product	Rate
13/9/94	0N 60P 60K	-
23/3/95	30 N 13.2 SO_3	-
21/4/95	Ammonium nitrate prills	87.4 Kg N ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
20/3/95	NEW 5C CYCOCEL (Chlormequat + Choline Chloride)	1.75 l ha^{-1}
13/4/95	NEW 5C CYCOCEL (Chlormequat + Choline Chloride)	2.5 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
12/4/95	SPORTAK DELTA (Prochloraz +Cyproconazole)	1.25 l ha^{-1}
5/5/95	FOLICUR (Tebuconazole)	1.0 l ha^{-1}
18/5/95	FOLICUR (Tebuconazole)	1.0 l ha^{-1}
2/6/95	RADAR (Propiconazole)	0.5 l ha^{-1}

Herbicide Application

Date	Product	Rate
21/9/94	GRAMOXONE (Paraquat)	2.0 l ha^{-1}
25/11/94	SABRE (Isoproturon) PANTHER (Diflufenican + Isoproturon)	3.75 l ha^{-1} 0.5 l ha^{-1}

Insecticide application

Date	Product	Rate
5/5/95	APHOX (Pirimicarb)	0.28 kg ha^{-1}
2/6/95	DECIS (Deltamethrin)	0.25 l ha^{-1}
14/6/95	AMBUSH C (Cypermethrin)	0.25 l ha^{-1}

2 J : Crop Husbandry - ADAS Bridgets : Field : Arizona 1995/1996

Sown - 17 th October 1995

Seed bed Preparation - Ploughed and presse

Soil Status (Tested 1995)

Soil type	pH	K^+	Mg^{2+}	P	Residual soil Nitrogen
Silty clay loam	8.1	129 mg l^{-1}	41 mg l^{-1}	16 mg l^{-1}	-

Nitrogen and Manganese Application

Date	Product	Rate
15/8/95	0N 40P 60K	-
16/3/96	30N 2P 33SO_3	-
2/5/96	Ammonium nitrate prills	46 Kg N ha^{-1}

Plant Growth Regulator Application

Date	Product	Rate
20/3/95	CYCOCEL (Chlormequat + Choline Chloride)	2.5 l ha^{-1}
13/4/95	MODDUS (Trinexapac-ethyl)	0.2 l ha^{-1}

Fungicide Application

Date	Product	Rate
Seed treatment	RAPPOR (Guazatine)	2 ml kg^{-1}
5/4/96	FOLICUR (Tebuconazole)	0.5 l ha^{-1}
2/6/96	BRAVO (Chlorothalonil) OPUS (Tebuconazole)	1.0 l ha^{-1} 1.0 l ha^{-1}
12/6/96	PATROL (Propiconazole)	0.5 l ha^{-1}
12/6/95	FOLICUR	0.5 l ha^{-1}

Herbicide Application

Date	Product	Rate
7/3/96	PANTHER (Diflufenican + Isoproturon) + IPU (Isoproturon)	2.0 l ha^{-1} 3.0 l ha^{-1}

Insecticide application

Date	Product	Rate
5/5/95	DURSBAN 4 (Chlorpyrifos)	0.28 kg ha^{-1}
2/6/95	DECIS (Deltamethrin)	0.03 l ha^{-1}

Appendix : 3

3A : Rates of development (Degree days after ear emergence, Date, Zadoks growth stage)of Haven at Harper Adams between 1994-1996

1994					1995					1996				
Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS
0	-	16 Jun	0	55	0	-	09 Jun	0	55	0	-	17 Jun	0	55
123	0	24 Jun	8	65	135	0	20 Jun	11	65	115	0	26 Jun	9	65
218	95	01 Jul	15	71-73	300	165	30 Jun	21	71-73	298	183	11 Jul	24	75
319	196	06 Jul	20	71-73	396	261	06 Jul	27	73	385	270	17 Jul	30	77
424	301	12 Jul	26	75	493	358	11 Jul	32	77	506	391	24 Jul	37	77
536	413	19 Jul	33	75-77	630	495	19 Jul	40	77-83	597	482	30 Jul	43	83
649	526	25 Jul	39	77	731	596	25 Jul	46	83-85	736	621	08 Aug	52	85
736	613	30 Jul	44	83	830	695	30 Jul	51	85-92	797	682	12 Aug	56	85-87
829	706	04 Aug	49	83-85	962	827	05 Aug	57	87-92	919	804	19 Aug	63	87
912	789	09 Aug	54	87-92	1038	903	10 Aug	62	92	1048	933	27 Aug	71	87-92
1008	885	16 Aug	61	87-92	*	*	*	*	*	1126	1011	02 Sep	77	92
1050	927	19 Aug	64	92	*	*	*	*	*	*	*	*	*	*

3B : Rates of development (Degree days after ear emergence, Date, Zadoks growth stage) of Hornet at Harper Adams between 1994-1996

1994					1995					1996				
Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS
0	-	15 Jun	0	55	0	-	09 Jun	0	55	0	-	17 Jun	0	55
119	0	23 Jun	8	65	118	0	19 Jun	10	65	102	0	25 Jun	8	65
206	87	28 Jun	13	71-73	300	182	30 Jun	21	71-73	298	196	11 Jul	24	75
323	204	05 Jul	20	73-75	397	279	06 Jul	27	73	385	283	17 Jul	30	77
423	304	11 Jul	26	75	490	372	11 Jul	32	77	506	404	24 Jul	37	77
540	421	18 Jul	33	75-77	657	539	20 Jul	41	77-83	597	495	30 Jul	43	83
647	528	24 Jul	39	77	747	629	25 Jul	46	83-85	736	634	08 Aug	52	85
736	617	29 Jul	44	83	849	731	30 Jul	51	85-92	797	695	12 Aug	56	85-87
827	708	03 Aug	49	83	980	862	05 Aug	57	87-92	919	817	19 Aug	63	87
914	795	08 Aug	54	87	1056	938	10 Aug	62	92	1048	946	27 Aug	71	87-92
1012	893	15 Aug	61	87-92	*	*	*	*	*	1126	1024	02 Sep	77	92
1069	950	19 Aug	65	92	*	*	*	*	*	*	*	*	*	*

3C : Rates of development (Degree days after ear emergence, Date, Zadoks growth stage) of Pastiche at Harper Adams between 1994-1996

1994					1995					1996				
Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS
0	-	14 Jun	0	55	0	-	07 Jun	0	55	0	-	16 Jun	0	55
132	0	23 Jun	9	65	141	0	19 Jun	12	65	120	0	25 Jun	9	65
219	87	28 Jun	14	73	303	162	29 Jun	22	71-73	298	178	10 Jul	24	75
336	204	05 Jul	21	73-75	418	277	06 Jul	29	73	390	270	16 Jul	30	77
436	304	11 Jul	27	75	516	375	11 Jul	34	77	506	386	23 Jul	37	77
553	421	18 Jul	34	75-77	653	512	19 Jul	42	77-83	595	475	29 Jul	43	83
660	528	24 Jul	40	77-83	721	580	23 Jul	46	83-85	734	614	07 Aug	52	85
749	617	29 Jul	45	83-85	795	654	27 Jul	50	83-87	815	695	12 Aug	57	85-87
840	708	03 Aug	50	85	985	844	05 Aug	59	87-92	937	817	19 Aug	64	87
927	795	08 Aug	55	87-92	1061	920	10 Aug	64	92	1066	946	27 Aug	72	87-92
1025	893	15 Aug	62	92	*	*	*	*	*	1144	1024	02 Sep	78	92
1082	950	19 Aug	66	92	*	*	*	*	*	*	*	*	*	*

3D : Rates of development (Degree days after ear emergence, Date, Zadoks growth stage) of Riband at Harper Adams between 1994-1996

1994					1995					1996				
Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS	Deg days after ZGS 55	Deg days after ZGS 65	Date	Days after ZGS 55	ZGS
0	-	15 Jun	-	55	0	-	07 Jun	-	55	0	-	16 Jun	-	55
119	0	23 Jun	8	65	126	0	18 Jun	11	65	108	0	24 Jun	8	65
206	87	28 Jun	13	73	303	177	29 Jun	22	71-73	298	190	10 Jul	24	75
323	204	05 Jul	20	73-75	418	292	06 Jul	29	73	390	282	16 Jul	30	77
423	304	11 Jul	26	75-77	516	390	11 Jul	34	77	506	398	23 Jul	37	77
540	421	18 Jul	33	75-77	653	527	19 Jul	42	77-83	595	487	29 Jul	43	83
628	509	23 Jul	38	77	721	595	23 Jul	46	83-85	734	626	07 Aug	52	85
736	617	29 Jul	44	83-85	795	669	27 Jul	50	83-87	815	707	12 Aug	57	85-87
827	708	03 Aug	49	83-85	919	793	02 Aug	56	87	937	829	19 Aug	64	87
914	795	08 Aug	55	87-92	1061	935	10 Aug	64	92	1066	958	27 Aug	72	87-92
1012	893	15 Aug	62	92	*	*	*	*	*	1144	1036	02 Sep	78	92
1069	950	19 Aug	66	92	*	*	*	*	*	*	*	*	*	*

Appendix : 4

Related studies undertaken in the course of research :-

I attended the following crop physiology / agricultural science related events :-

1) Cereals 1994 "Growing for Quality", Thriplow, Cambridge (15/6/94).
2) ADAS Conference, "Quality Cereal Production", Steeple Aston, Oxon (15/7/94).
3) H-GCA Agronomy Update 1994, Harper Adams Agricultural College (17/11/94).
4) Brighton Crop Protection Conference : Pests and Diseases 1994 (21-24/11/94).
5) The Fifth H-GCA Cereals Research and Development Conference, Robinson College, Cambridge (10/1/95).
6) University of Nottingham/ADAS/HAAC Agronomy Group Workshop Seminars, Mildenhall, Cambridge (26-27/1/95).
7) Cereals 1995 "Efficiency through Effective Management", Shuttleworth Agricultural College, Bedfordshire (14/6/95).
8) NIAB Cereals West, Harper Adams Agricultural College (22/6/95).
9) H-GCA Agronomy Update 1995, Harper Adams Agricultural College (14/11/95).
10) Association of Independent Crop Consultants Technical Information Update, Sutton Bonington, University of Nottingham (7/12/95).
11) Cereals 1996 "The National Cereals and Combinable Crops Event" Aubourn Farming, The Nevile Estate, Wellingore, Lincoln (13/6/96).
12) H-GCA Agronomy Update 1996, Harper Adams Agricultural College (8/11/96).
13) Brighton Crop Protection Conference : Pests and Diseases 1996 (18-21/11/96).

I undertook the following training courses :-

1) Air-segmented flow autoanalyser training at Skalar UK Ltd., York (2-3/6/94).
2) NPTC training and testing PA1 (20/6/94).
3) NPTC training and testing PA6A (21/6/94).
4) NAg Genstat 5 Release 3.3 Introductory Course (11-12/4/95).
5) GCSE French, Telford Technical College (Sept 95 - June 96).
6) Quality Systems and Instrumentation for Cereal Processors, Continuing Professional Development Course, The Satake Centre for Grain Process Engineering, The Manchester Institute of Science and Technology (9-10/6/97).
7) BBSRC Technical Writing Course, Dr David Cooke, IACR-Brooms Barn (8-9/9/97).

I gave the following presentations :-

1) Poster displayed at 7 th International Symposium on Pre-Harvest Sprouting in Cereals. Japan (2-7/7/95).
2) Departmental Seminar "Environmental factors affecting *alpha*-amylase activity and Hagberg falling number in wheat" (29/2/96).
3) Poster presented at The Society for Experimental Biology, Annual Meeting, University of Lancaster (25-29/3/96).
4) Poster presented at First European Symposium on Enzymes in Grain Processing,

Leewenhorst Congress Centre, Noordwijkerhout, Netherlands (2-4/12/96).

5) Poster presented at SCI Conference, "Growing Cereals for Quality", London (19/11/96).
6) Presentation at Cereals 1997, The Arable Farming Event on "Preharvest Hagberg falling number determination" (11-12/6/97).
7) Presentation at University of Nottingham / ADAS Research Centre, Agronomy Workshop "The origin of *alpha*-amylase in wheat grain : Pre-maturity activity in the absence of sprouting" (25-26/9/97).
8) Presentation at Harper Adams Postgraduate Student Colloquium : Environmental factors affecting pre-maturity *alpha*-amylase activity in winter wheat (13/10/97).
9) Poster presented at 8 th International Symposium on Pre-harvest Sprouting in Cereals, Detmold, Germany (1-5/6/98).
10) Daily Telegraph Young Science Writer Competition Runner-Up : "Flattened toads and Suet Pudden" (16/8/98).

Appendix : 5

Statement concerning contributions to this thesis made by others.

This thesis has formed part of a larger research project. The inclusion of some of the data included in Section 3 of this thesis has been made possible by the contribution of other research workers. This is detailed in the list below. All statistical analysis, interpretation and presentation of data in Section 3 has been undertaken solely by the author.

Section 3.2.2 - The choice of cultivars and the initial experimental design of the field experiment at HA in 1993/4 was made by Mr Peter Kettlewell. Subsequent experimental designs employed at the field sites at AB, SB and UA were devised by field site managers.

Section 3.2.5 - Sampling methods employed at AB, HA, SB and UA were based on a preliminary protocol devised in collaboration with Dr Gavin Lunn at the University of Nottingham. Collaborating workers at the research sites were responsible for sample collection, and some processing of samples. At HA, research assistants were responsible for approximately 50 % of the samples collected and processed.

Section 3.2.6 + 3.2.7 - Collaborators at UA, AB and SB, recorded weather data and plant development stages at these sites.

Section 3.2.9 - Moisture content measurements for separate sites were undertaken by collaborators at UA, AB and SB. At HA approximately 50 % of the moisture content measurements were undertaken by research assistants.

Section 3.2.15 - The initial *beta*-limit dextrin gel and iodine staining test was devised by Kalpna Kotecha at CCFRA, who used this method to analyse samples from HA in 1994 (part of Table 3.16).

Section 3.2.16 - The initial Phadebas gel assay was devised by Rachel Seamer at CCFRA.

Table 3.16 - Approximately 75 % of the FDB assays were undertaken by research assistants at HA, with 75 % of the gel assays also undertaken by research assistants at HA.

Section 3.3.5.2 - *Alpha*-amylase assays on samples from HA 1995 and SB 1995 were undertaken by a research assistant at HA.

Table 3.17 + Table 3.18 - Approximately 50 % of all the HFN determinations were undertaken by research assistants at HA.